Quercetin Induces Apoptosis via Caspase Activation, Regulation of Bcl-2, and Inhibition of PI-3-Kinase/Akt and ERK Pathways in a Human Hepatoma Cell Line (HepG2)¹

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Abstract

Dietary polyphenols have been associated with the reduced risk of chronic diseases such as cancer, but the precise underlying mechanism of protection remains unclear. The aim of this study was to investigate the effect of quercetin on the activation of the apoptotic pathway in a human hepatoma cell line (HepG2). Treatment of cells for 18 h with quercetin induced cell death in a dose-dependent manner; however, a shorter treatment (4 h) had no effect on cell viability. Incubation of HepG2 cells with quercetin for 18 h induced apoptosis by the activation of caspase-3 and -9, but not caspase-8. Moreover, this flavonoid decreased the Bcl-xL:Bcl-xS ratio and increased translocation of Bax to the mitochondrial membrane. A sustained inhibition of the major survival signals, Akt and extracellular regulated kinase (ERK), also occurred in quercetin-treated cells. These data suggest that quercetin may induce apoptosis by direct activation of caspase cascade (mitochondrial pathway) and by inhibiting survival signaling in HepG2. J. Nutr. 136: 2715–2721, 2006.

Introduction

Hepatocellular carcinoma (HCC)² is a global health problem and the third-ranked cause of cancer-related deaths in the world (1). HCC is difficult to detect and in most cases is not noticed at an early enough stage, which, together with an HCC annual occurrence in more than a half-million people (2) and 600,000 deaths in 2002 (1), creates an important reason for searching chemotherapeutic/chemopreventive agents for HCC.

Epidemiological and intervention studies in both humans and animals have shown that regular consumption of fruits, vegetables, and tea is associated with reduced risk of cancer (3,4). Fruits, vegetables, spices, and tea provide essential nutrients and many diet-derived phenolics, in particular flavonoids, which have been reported to exert potential anticarcinogenic activities (3–6). Quercetin is one of the most common flavonoids found in many diet-derived phenolics, in particular flavonoids, which have been reported to exert potential anticarcinogenic activities (3–6). Quercetin is one of the most common flavonoids found in the diet (4) and is extensively metabolized during absorption in the small intestine and in the liver (7). Recent studies have shown that quercetin has antiproliferative effects (5,8) and can induce DNA fragmentation in other human hepatocarcinoma cell lines (6), leukemia (21), and mouse thymocytes (22). Quercetin also downregulates anti-apoptotic proteins of the Bcl-2 family, Bcl-xL and Bcl-2 (23,24), and upregulates pro-apoptotic members, Bax and Bad (23,24). Additionally, apoptosis may also be induced by oxidative stress commonly associated with a previous increase of intracellular reactive oxygen species (ROS), which can act as signaling molecules to trigger apoptosis under certain situations (25,26). Alternatively, the modulation of signaling through the serine/threonine kinase, Akt/protein kinase B (PKB), phosphatidylinositol-3-kinase (PI-3-kinase), and members of the mitogen-activated protein kinase (MAPK) family, such as extracellular regulated kinase (ERK), may also be relevant in the molecular mechanism of action of this flavonoid. In this regard, quercetin modulates the enzymes involved in proliferation and signal transduction pathways, including members of the MAPK family,

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² Abbreviations used: ERK, extracellular regulated kinase; HCC, hepatocellular carcinoma; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; PI-3-kinase, phosphatidylinositol-3-kinase; PKB, protein kinase B; ROS, reactive oxygen species.

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such as ERK and c-Jun N-terminal kinase (JNK) (27,28), and to inhibits PI-3-kinase (10,21,29) and Akt (11,27,28).

The present study investigates the mechanisms underlying the cytotoxic effect of quercetin on a human hepatoma cell line (HepG2), assessing its influence on the balance between-prodeath pathways, such as apoptotic cascade through caspases (caspases-3, -8, and -9), and some Bcl-2 family members (Bcl-xL and Bax) and prosurvival pathways, namely, Bcl-2, Akt/PI-3-kinase, and ERK.

Materials and Methods

Materials and chemicals. Quercetin, trypan blue, piruvate, NADH, gentamicin, penicillin G, and streptomycin were purchased from Sigma Chemical. The fluorescent probe 2,7’-dichlorofluorescin diacetate (DCFH-DA) was from Molecular Probes. Antiactive cleaved caspase-3, antiactive caspase-9, anti-Akt and antiphospho-Ser473-Akt, as well as anti-ERK1/2 and antiphospho-ERK1/2 recognizing phosphorylated Thr202/Thr204 of ERK1/2, and anti-β-actin were obtained from Cell Signaling Technology (sc-634, sc-526 and sc-7175, respectively). Anti-p88α/β was from Upstate NY Biotechnology. Caspase-3 and caspase-8 substrates (M Ac-DEVD-AMC and Ac-IETD-AMC, respectively) were purchased from Pharmingen. Materials and chemicals for electrophoresis were from BioRad. Cell culture dishes and cell culture medium were from Falcon and Biowhittaker Europe (Innogenetics), respectively.

Cell culture and quercetin treatments. Human hepatoma HepG2 cells were a gift from Dr. Paloma Martin-Sanz (Centro Nacional de Investigaciones Cardiovasculares, Madrid). They were grown in DMEM-F12 medium supplemented with 2.5% fetal bovine serum and the following antibiotics: gentamicin, penicillin, and streptomycin (50 mg/L). Cells were maintained in 37°C in a humidified atmosphere of 5% CO2. Cells were seeded and routinely grown in DMEM-F12 medium and 2.5% fetal bovine serum, but they were changed to serum-free medium 24 h before the assay. Cells were treated with different concentrations of quercetin (10, 25, 50, 75, and 100 μmol/L) during 4 or 18 h.

Viability cell assay. Viability was calculated by counting the cells in a Neubauer chamber. An aliquot of the total cell suspension (1.5 x 10^6 cells) was mixed with an equal volume of trypan blue and incubated for 5 min at room temperature.

Cytotoxicity assay. Lactate dehydrogenase leakage assay (LDH) was carried out as previously described (30,31). In brief, culture medium was collected separately and the cells were scraped. Cell suspension (1.5 x 10^6 cells) was sonicated to ensure breaking down the cell membrane to release the total amount of LDH. A mixture of 5 mmol/L pyruvate, 0.35 mmol/L NADH, and 84 mmol/L Tris was added to the sample and read at 340 nm in a microplate ELISA reader (Bio-Rad). LDH leakage was estimated as the ratio between the LDH activity in the culture medium and that of the whole cell content.

Determination of ROS. Cellular oxidative stress was quantified by the dichlorofluorescin (DCF) assay using a microplate reader (30,32). After being oxidized by intracellular oxidants, DCF becomes dichlorofluorescein (DCF) and emits fluorescence. By quantifying fluorescence at an excitation wavelength of 485 nm and emission wavelength of 530 nm, a fair estimation of the overall oxygen species generated under the different conditions was obtained.

Purification of mitochondrial and cytosolic extracts. Cells were incubated in a hypotonic buffer [1 mmol/L EDTA, 10 mmol/L HEPES, 50 mmol/L sucrose (pH 7.6)] at 37°C and homogenized. Then, after the addition of a hypertonic solution [1 mmol/L EDTA, 10 mmol/L HEPES, and 450 mmol/L sucrose (pH 7.6)], a supernatant was obtained corresponding to the cytosolic fraction and a pellet with the mitochondria.

Mitochondria were resuspended in an isotonic buffer [1 mmol/L EDTA, 10 mmol/L HEPES, and 250 mmol/L sucrose (pH 7.6)].

Western blot analysis. Total cell extracts were obtained as previously described (33) except for the analysis of the levels of Akt, phospho-Akt, ERK1/2, phospho-ERK1/2, and PI-3-kinase subunits (p85 and p110), which were prepared according to Fabregat et al. (34).

Equal amounts of proteins (25–100 μg) were separated by SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride filters (PVDF, Protein Sequencing Membrane, Bio-Rad) that were probed with the corresponding primary antibody. Western blots were developed with the ECL system (GE Healthcare). Normalization was ensured by β-actin and bands were quantified by laser scanning densitometry (Molecular Dynamics).

Fluorometric analysis of caspase-3 and -8 activities. Caspase-3 and -8 activities were measured as previously described (33). Briefly, cells were lysed in a buffer containing 5 mmol/L Tris (pH 8), 20 mmol/L EDTA, and 0.5% Triton-X100. For caspase-3 activity, reaction mixture contained 20 mmol/L HEPES (pH 7), 10% glycerol, 2 mmol/L dithiothreitol, 30 μg protein per condition, and 20 μmol/L Ac-DEVD-AMC (N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin) as substrate. Reaction mixture for caspase-8 activity contained 20 mmol/L piperase, N,N’-bis(2-ethyl sulfonic acid) (pH 7.2), 100 mmol/L NaCl, 10 mmol/L dithiothreitol, 1% EDTA, 0.1% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate, 10% sucrose, 70 μg protein/condition, and 22.6 μmol/L Ac-IETD-AMC (N-acetyl-Leu-Glu-Thr-Asp-7-amino-trifluoromethylcoumarin) as substrate. Enzymatic activity was measured at excitation wavelength of 380 nm and emission wavelength of 440 nm.

Statistics. Statistical analysis was as follows: data were analyzed using 2-way ANOVA with concentration and time as the 2 factors tested. Significant time x concentration interactions were found for all variables, and subgroups were analyzed further by testing the effect of the concentration within each group using 1-way ANOVA. Data were tested prior to analysis for homogeneity of variances using Levene’s test. In experiments where only one factor was studied (i.e., concentration), data were evaluated using 1-way ANOVA followed by the Bonferroni test when variances were homogeneous or by the Tamhane test when variances were not homogeneous (LDH and ROS, 18-h treatment). Differences with P < 0.05 were considered significant. The SPSS version 12.0 program was used.

Results

Cell viability and intracellular generation of ROS. To determine the accurate range of cytotoxic concentrations of quercetin in a human hepatoma cell line (HepG2) we used 2 different methods to assay cell viability, trypan blue exclusion and LDH leakage. Cells were exposed to 0–100 μmol/L of quercetin for 4 or 18 h. Quercetin induced cell death in a dose-dependent manner after 4 or 18 h of incubation, as determined by the trypan blue exclusion assay (Table 1). Incubation with quercetin for 18 h displayed a dramatic cell mortality (68% with the highest concentration, 100 μmol/L, P < 0.05) with an estimated 50% of cell death (IC50) value of 87 μmol/L (Table 1). In contrast, treatment for 4 h slightly but significantly reduced the cell viability compared with controls (up to 10% at the highest concentration, 100 μmol/L, P < 0.05).

Intracellular lactate dehydrogenase (LDH) released to the culture medium was also used as an indicator of cytotoxicity. After 4 h of treating HepG2 cells with quercetin, no cytotoxic effects occurred at any concentration (Table 1). In contrast, 18 h of treatment with 10–100 μmol/L quercetin increased LDH activity in the cell culture medium, indicating cell damage. It is remarkable that the highest leakage of LDH to the medium occurred at 50 μmol/L quercetin and was significantly less at higher concentrations (75 and 100 μmol/L).
We assayed ROS production to test whether different concentrations of quercetin had an effect on the generation of oxygen radicals in HepG2 in culture. Quercetin at 10–75 μmol/L enhanced ROS generation after 4 h treatment but did not have an effect at 100 μmol/L (Table 1). Interestingly, at 18 h, intracellular ROS levels were significantly decreased in the presence of 10–100 μmol/L quercetin at 18 h in a dose-dependent fashion. Leakage of probe was not observed in cells throughout the assay, as determined in previous tests during method set-up in our laboratory (35). Thus, any potential contribution of extracellularly oxidized DCF to the final fluorescence can be ruled out.

### Caspase processing in the induced apoptosis

We tested the effect of quercetin on the cascade of caspases that are crucial initiators or effectors in the cell death pathways. Enzymatic activity of caspase-8 was unchanged after 4 or 18 h of incubation at all quercetin concentrations (data not shown). A significant activation of caspase-3 occurred at 25 μmol/L quercetin after 18 h of incubation. Activity was greater at 50 μmol/L quercetin but decreased at 75 and 100 μmol/L (Table 2). Proteolytic caspase-3 activity was not affected by any quercetin concentration after 4 h treatment (Table 2) and increased only slightly (P = 0.05) due to 50 μmol/L after 8 h (data not shown). The activation of caspase-3 after 18 h was confirmed by Western blot analysis (Fig. 1). Parallel to the cytotoxic effect and the enhanced caspase-3 activity, treatment of HepG2 cells with quercetin for 18 h increased the levels of active caspase-3; in fact, the cleaved caspase-3 level increased (P < 0.05) after 18 h of treatment with 25 μmol/L quercetin (Fig. 1A and 1B), and increased further at higher concentrations, especially at 50 μmol/L. Consistent with our previous results (19), the short-term treatment (4 h) did not have a prominent cytotoxic effect nor did it greatly induce apoptosis (data not shown). Therefore, the remaining assays were conducted using the 18-h quercetin treatment.

Active caspase-9 appeared after 18 h incubation with 25 μmol/L quercetin (Fig. 1), being caspase-9 processing coincident with caspase-3 activation. Moreover, caspase-9 (zymogen) expression levels increased at 25–75 μmol/L quercetin concentrations.

### Anti-apoptotic and pro-apoptotic Bcl-2 family members

The balance of the expression of anti- and pro-apoptotic members of the Bcl-2 gene family is one of the major mechanisms that regulates apoptosis in mammalian cells (36). Therefore, to determine whether quercetin-induced apoptosis in HepG2 was also associated with the modulation of members of this protein family, we examined the expressions of Bcl-x and Bax.

Bcl-xL has been proposed to be a caspase substrate, and the product of Bcl-xL cleavage, Bcl-xS, has a pro-apoptotic function. This proteolytic fragment (Bcl-xS) was detected when the cells were incubated for 18 h and the levels were greater at all quercetin concentrations compared with controls (Figs. 2A). The Bcl-xS:Bcl-xL ratio was decreased by quercetin, which reached a minimum value at 50 μmol/L (Figs. 2A and 2B).

Bax has been shown to translocate from the cytoplasm to the outer mitochondrial membrane to form pores and mediate the apoptotic death (36). Mitochondrial and cytoplasmatic fractions were evaluated by Western blot, and we observed that after treatment with quercetin for 18 h, translocation of Bax to the mitochondria increased to its highest level at 50 μmol/L, decreased at 75 μmol/L, and reached control levels at 100 μmol/L (Figs. 2A and 2C); as expected, the opposite pattern occurred in the cytosolic fraction (Figs. 2A and 2D).

### Akt phosphorylation and PI-3-kinase protein levels

Phosphorylation activation of Akt is associated with protection of cells from apoptosis (37). To analyze whether inhibition of Akt phosphorylation is related to quercetin-induced apoptosis, we measured total and phosphorylated levels of this protein. Concentrations of quercetin above 50 μmol/L inhibited Akt by decreasing the level of phosphorylated active Akt and, in contrast, lower concentrations of quercetin (10 and 25 μmol/L) resulted in the activation of Akt (Figs. 3A and 3B).

The PI-3-kinase pathway is regulated by a variety of growth factors, and the activation of the PI-3-kinase/Akt signaling pathway is strongly implicated in the regulation and survival or protection of cells (37). Thus, we also examined the effect of quercetin on the levels of the p85 regulatory subunit and the p110 catalytic subunit of PI-3-kinase. The expression of both subunits was not altered by quercetin treatment (Fig. 3).

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**TABLE 1** Effect of quercetin on viability, LDH leakage, and intracellular ROS in HepG2 cells

<table>
<thead>
<tr>
<th>Quercetin (μmol/L)</th>
<th>Viability</th>
<th>LDH leakage</th>
<th>ROS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% dead cells</td>
<td>% LDH</td>
<td>fluorescence units</td>
</tr>
<tr>
<td>0</td>
<td>2.53 ± 0.70*</td>
<td>4.10 ± 1.02*</td>
<td>1260 ± 68a</td>
</tr>
<tr>
<td>10</td>
<td>10.56 ± 0.51bc</td>
<td>1.99 ± 0.31a</td>
<td>2803 ± 419b</td>
</tr>
<tr>
<td>25</td>
<td>12.96 ± 1.19c</td>
<td>1.64 ± 0.23a</td>
<td>1075 ± 119b</td>
</tr>
<tr>
<td>50</td>
<td>15.57 ± 3.40c</td>
<td>2.60 ± 1.00a</td>
<td>721 ± 101c</td>
</tr>
<tr>
<td>75</td>
<td>35.69 ± 8.95d</td>
<td>3.70 ± 1.09a</td>
<td>682 ± 29Fd</td>
</tr>
<tr>
<td>100</td>
<td>69.72 ± 4.74e</td>
<td>1.84 ± 1.00a</td>
<td>643 ± 34F</td>
</tr>
</tbody>
</table>

*Values are means ± SEM, n = 8–10 (viability), n = 12 (ROS) or n = 6–8 (LDH leakage). Time × concentration interaction was significant, P < 0.05. Means in a column without a common letter differ, P < 0.05.

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**TABLE 2** Effect of quercetin on caspase-3 activity.

<table>
<thead>
<tr>
<th>Quercetin (μmol/L)</th>
<th>Caspase-3 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of incubation</td>
</tr>
<tr>
<td>0</td>
<td>4 h</td>
</tr>
<tr>
<td>10</td>
<td>18 h</td>
</tr>
<tr>
<td>25</td>
<td>34.61 ± 1.74a</td>
</tr>
<tr>
<td>50</td>
<td>33.60 ± 1.11a</td>
</tr>
<tr>
<td>75</td>
<td>33.57 ± 0.99a</td>
</tr>
<tr>
<td>100</td>
<td>31.65 ± 0.47a</td>
</tr>
</tbody>
</table>

*Values are means ± SEM, n = 8. Time × concentration interaction was significant, P < 0.05. Means in a column without a common letter differ, P < 0.05.
Extracellular regulated kinase phosphorylation. The ERK signaling pathway is activated in response to certain situations of cellular stress, and it is implicated in cellular death or survival signaling (38). Therefore, we investigated whether quercetin-induced apoptosis was related to ERKs. Quercetin evoked a dose-dependent inhibitory effect in the 2 bands corresponding to ERK1 and ERK2 at all concentrations that induced apoptosis (Fig. 4). Similar to Akt regulation, treatment of HepG2 cells for 18 h with quercetin enhanced the phosphorylation of ERK1/2 at 10 μmol/L and dephosphorylation at concentrations above 50 μmol/L.

Discussion

Epidemiological studies have demonstrated the relation between diet and cancer and the potential of dietary components, in particular, polyphenols, as antiproliferative agents (3–6). Targets for chemoprevention in carcinogenesis could be multiple (3,4), and induction of apoptosis is becoming an important preventive approach. In this context, a continuous daily intake of polyphenols like quercetin might result in steady-state plasmatic concentrations, even in levels that might provoke an apoptotic cell death (10–50 μmol/L), which has been observed after the intake of dietary supplements (39). Our study showed that quercetin modulates apoptosis induction in HepG2 cells; that is, it stimulates proteolytic activities of caspase-3 and -9, alters the expression of some of the Bcl-2 family of proteins, and dephosphorylates Akt and ERK1/2.

Quercetin concentrations that significantly decrease HepG2 cell viability have similar effects on other cancer cell cultures, such as leukemia (9), human colon (8), prostatic (24,40), breast (10), lung (11) cancer cells, and murine hepatoma (12). Our result with quercetin further demonstrates the inhibitory effect of this flavonoid on tumor cell viability. Increased leakage of LDH from the cell was unexpectedly observed upon exposure to common reducing agents such as glutathione or ascorbate (41) and also after long quercetin treatments (30,42); however, decreased activity of LDH by oxidation of essential enzyme
residues in the presence of metals, light, or various oxidants (i.e., hydrogen peroxide) has also been demonstrated (43). In this context, we observed that LDH leakage could be affected by quercetin, and yet we observed no inhibition of this enzyme activity by this flavonoid (data not shown).

ROS are highly reactive metabolites generated during normal cell metabolism; however, elevated intracellular ROS could be sufficient to trigger apoptosis (25,26). Moreover, apoptotic cell death is reported to be preceded by the following sequential facts: ROS production, loss of mitochondrial transmembrane potential, release of cytochrome c, and activation of caspase-3 in fetal rat hepatocytes (33). Our previous (19) and present results (4-h treatment) attribute the enhanced production of ROS to the initial oxidative stress prior to the apoptotic process, although quercetin might not require enhanced ROS production for the apoptosis induction, as previously described for TGF-β1 in fetal rat hepatocytes (44) or IL-3 in murine myeloid cells (45). However, inhibition of ROS after 18 h of flavonoid treatment suggests that quercetin at high concentrations or long exposures might inhibit the mitochondrial respiratory chain (46). This feature, supported by present and previous results (19), could be related either to apoptosis or necrosis, and therefore, a small percentage of necrosis cannot be ruled out.

Caspase-8 was not activated in response to the quercetin treatment, indicating that the extrinsic pathway was not involved in quercetin-induced caspase-3 activation and in apoptosis. Several studies indicate the activation of the mitochondrial pathway, and therefore caspase-9, by quercetin in leukemia (16) and colon cancer cells (8); similarly, caspase-9 was also cleaved by other polyphenols such as luteolin in a rat hepatoma cell line (47). In our study, caspase-9 processing was coincident with caspase-3 activation, which indicates that the highest activity of caspases implicated in the mitochondrial apoptosis pathway was found 18 h after treatment with 50 μmol/L quercetin.

As our results showed, expression of Bcl-xL, Bcl-xS, and Bax could be differently regulated by quercetin, suggesting that the balance in the expression of these proteins might be involved in the control of the apoptotic process. Quercetin decreased the Bcl-xL:Bcl-xS ratio; thus, regulation of Bcl-xL protein levels seems to be at least in part, caspase-dependent in HepG2 cells, which agrees with previous results in fetal hepatocytes treated with TGF-β1 (33). Herrera et al. (33) proposed that Bcl-xL is a caspase substrate. Quercetin also increased Bax translocation from the cytosol to the mitochondrial membrane, an event that promotes apoptotic death (44) and followed a similar pattern to caspase-3 and -9 activation and Bcl-xL:Bcl-xS decrease. Accordingly, increased levels of total Bax occurred in other studies of fetal hepatocytes (44) as well as in studies of human lung (11), prostate (24), and liver (23) cancer cells. Increased Bax translocation was also observed in HepG2 cells after luteolin treatment (48).

Akt promotes cell survival by inhibiting apoptosis, and its phosphorylation has been considered a critical factor in the aggressiveness of HCC (49). Quercetin induced inactivation of Akt by decreasing the level of phosphorylated Akt in a concentration-dependent manner, contributing to the promotion of apoptosis. Although the precise anti-apoptotic effects of Akt are still unclear, Akt directly phosphorylates and inactivates procaspase-9 and blocks caspase-9-mediated apoptosis (50).

This could explain the observed effects of low concentrations of quercetin (10 and 25 μmol/L) that induce a sharp increase in Akt phosphorylation levels (Fig. 3B) and accumulation of procaspase-9 (Fig. 1C) but did not result in increased levels of active caspase-3 and -9 and, consequently, showed reduced cytotoxic effects (Table 1). Alternatively, inhibition of Akt promotes
phosphorylation of the proapoptotic Bad, a fact that favors progress of the apoptotic process (1,49).

In contrast to the marked effects of quercetin on Akt phosphorylation, quercetin did not affect PI-3-kinase protein levels. This observation has been previously demonstrated in studies of human breast (10), lung (11), prostate (40) cancer cells, and of rat aortic smooth muscle cells (28). Quercetin and other flavonoids are reported to be PI-3-kinase inhibitors, reducing enzymatic PI-3-kinase activity without changing either p85 or p110 subunit levels (10,29,40), which agrees with the lack of effect of quercetin on PI-3-kinase levels observed in our study. Therefore, a reduction of PI-3-kinase activity by quercetin might be taking place, which might explain the observed decrease of Akt phosphorylation, its downstream target. PI-3-kinase activity will be investigated further in future studies.

Survival-signaling cascade in many cells involves PI-3-kinase, Akt, and also cross-communication between PI-3-kinase and ERKs (38). Because a sustained activation of ERK1/2 is necessary for cell survival and cell proliferation (38), the inhibition of ERK1/2 by quercetin contributes to the increased occurrence of cell death. However, quercetin exposure at low concentrations (10 μmol/L) results in an increased phosphorylation of ERK1/2, similar to what was observed for Akt, which suggests an activation of cell-survival pathways. Therefore, survival of HepG2 cells seems to depend on both ERK and PI-3-kinase/Akt pathways as previously reported in this cell line (51). A similar prosurvival effect of low quercetin concentrations (10 μmol/L) with increased phosphorylation of Akt and ERK was observed in neuronal cultures by Spencer et al. (27).

In summary, our studies showed that inhibition of Akt and ERK phosphorylation, induced by high quercetin concentrations, was coupled with a significant increase of caspase-3 and -9 levels and activities, higher expression of proapoptotic Bcl-2 family members (Bcl-xL and Bax), and lower levels of antiapoptotic Bcl-xL that contributed directly to the apoptotic process. Interestingly, the highest quercetin concentrations (75 and 100 μmol/L) reduced the expression of proapoptotic (caspase-3, -9, and Bax) and prosurvival signals (Akt and ERK) compared with lower concentrations of quercetin (50 μmol/L). Thus, the caspases implicated in the mitochondrial apoptosis pathway that showed the highest activity after 18 h of treatment with 50 μmol/L quercetin provoked the greatest activation of their Bcl-2 family substrates, whereas the apoptotic effect might have been enhanced by the inhibition of Akt and ERK. Higher concentrations further decreased cell viability due to the activation of executor apoptotic signal and the more pronounced inhibition of prosurvival pathways (Akt). Although special attention must be given to flavonoid concentrations, quercetin may be a potential chemopreventive or therapeutic agent in HCC, and further efforts to investigate these possibilities are needed.

Literature Cited

Mechanisms of quercetin-induced apoptosis