

## Combination of Quercetin and Kaempferol enhances in vitro Cytotoxicity on Human Colon Cancer (HCT-116) Cells

Sara Jaramillo-Carmona<sup>1,\*</sup>, Sergio Lopez<sup>2</sup>, Rocio Abia<sup>2</sup>,  
Rocio Rodriguez-Arcos<sup>1</sup>, Ana Jimenez<sup>1</sup>, Rafael Guillen<sup>1</sup>  
and Francisco J.G. Muriana<sup>2</sup>

<sup>1</sup> *Phytochemicals and Food Quality Group, Instituto de la Grasa (CSIC), 41014 Seville, Spain*

<sup>2</sup> *Laboratory of Cellular and Molecular Nutrition, Instituto de la Grasa (CSIC), 41012 Seville, Spain*

(Received April 15, 2013; Revised June 18, 2013; Accepted December 17, 2013)

**Abstract:** Colon cancer is one of the most common types of cancer malignancy. Although flavonoids naturally occur as mixtures, little information is available regarding the additive or synergistic biochemical interactions between flavonoids. The objectives of this study were to examine the feasibility of combining two major structurally related flavonoids, quercetin and kaempferol, to affect the cell viability, cell cycle, and proliferation of the human colon cancer HCT-116 cell line. The combination of quercetin and kaempferol exhibited a greater cytotoxic efficacy than did either quercetin or kaempferol alone. This effect was highest and acted in a synergistic fashion in a 2-fold quercetin and 1-fold kaempferol IC<sub>50</sub> combination, which also arrested cell growth in the G<sub>2</sub>/M phase and suppressed proliferation. Our observations support a structure-activity relationship based on the presence of 3'-OH moiety and/or 4'-OH moiety on the B-ring of flavonoids.

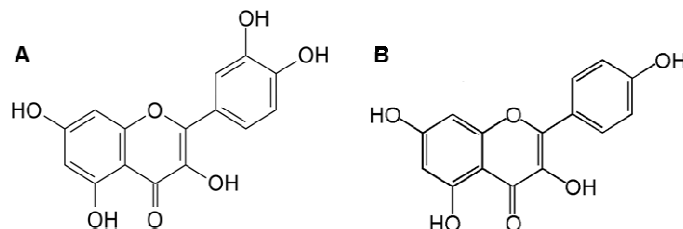
**Keywords:** Quercetin; kaempferol; colon cancer; cytotoxicity; synergy. © 2014 ACG Publications. All rights reserved.

### 1. Introduction

The increased consumption of vegetables and fruits significantly reduces the incidence of chronic diseases, such as cancer, cardiovascular diseases, and other age-related diseases [1]. Polyphenols, especially flavonoids, are thought to be the major bioactive compounds providing protections against these diseases. Humans' flavonoid intake is highly variable, with estimations ranging from 25 mg to more than 1 g per day [2]. The main sources of flavonoids are tea, onions, apples, broccoli, fresh kale, peaches, and spices, with the flavonoid quercetin being present in practically all plant-based diets and kaempferol being present in about 80% of diets [3]. Quercetin (3,3',4',5,7-pentahydroxyflavone) and kaempferol (3,4',5,7-tetrahydroxyflavone) exhibit minor differences in their structural characteristics, with quercetin having two -OH moieties on its B-ring, while kaempferol has only one (Figure 1). Numerous studies have shown that these flavonoids exhibit

\* Corresponding author: E-Mail: [smaria@cica.es](mailto:smaria@cica.es); Phone:0034-954611550 Fax:0034-954616790

anticancer activity by inducing cell cycle arrest and apoptosis and inhibiting proliferation in numerous types of cancer cell lines in leukemia, breast cancer, ovarian cancer, lung cancer, and colon cancer [4]. However, the beneficial effects of using quercetin and kaempferol in combination to achieve cytotoxicity in cancer cells remain poorly understood.



**Figure 1.** Structures of quercetin (A) and kaempferol (B).

Colon cancer represents almost 10% of all tumors. It is the third most common cancer in men in modern countries (after lung and prostate cancers) and the second in women (after breast cancer) with approximately 1 million new cases each year worldwide. Only 5-10% of these cases are due to genetic factors, while more than 70% are related to diet and lifestyle, suggesting that colon cancer rates could be substantially reduced by changes in dietary and lifestyle patterns [5]. Therefore, we investigated the *in vitro* chemotherapeutic potential of these flavonoids by testing the effect of quercetin and kaempferol in combination on the human colon cancer HCT-116 cell line.

## 2. Materials and Methods

### 2.1. Materials

All cell culture reagents were purchased from Gibco (Invitrogen, Grand Island, USA). Quercetin was obtained from Sigma-Aldrich (Saint-Louis, Missouri, USA) and kaempferol from Extrasynthese (Lyon, France). Stock solutions of each flavonoid were prepared in dimethyl sulfoxide (DMSO) and stored at  $-20^{\circ}\text{C}$ . Trypan blue, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), DMSO, Triton X-100, EDTA, RNase, and propidium iodide (PI) were purchased from Sigma-Aldrich.

### 2.2 Cell Line

The human colorectal carcinoma cell line HCT-116 was obtained from the American Type Culture Collection (Bethesda, USA; ATCC #CCL247). Cells were grown at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$  and 90% relative humidity in McCoy's 5A medium, supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100  $\mu\text{g}/\text{mL}$  streptomycin (complete medium). The cells were harvested using 0.25% trypsin-EDTA and plated to the required density. Under these conditions, HCT-116 cells display a poor degree of differentiation and a doubling time of approximately 36 h. All the experiments were performed with between 3 and 10 passages.

### 2.3 Cell Viability Assay

HCT-116 cells were plated at 50000 cells/well in 0.2 mL of medium for 24 h. Subsequently the cells were exposed to quercetin, kaempferol, and different combinations of the two in a complete medium for 48 h. The final concentration of DMSO in the culture medium was maintained at  $<0.1\%$ ,

which is a concentration that causes neither growth effects nor cell death within 48 h. The cell viability was assayed based on the ability of live cells to reduce MTT. After the flavonoid treatment, 0.02 mL of MTT reagent (5 mg/mL MTT in PBS) was added to each well. After 3 h, the medium was removed, and the cells were incubated for 30 min with 0.1 mL of DMSO. The concentration of formazan was measured spectrophotometrically at 490 nm using a Multiskan Spectrum microplate reader (Thermo Labsystems, Rockford, USA). All MTT assays were carried out in three separate.

#### *2.4 Synergy Determination*

After the flavonoid treatment, the data obtained with the MTT assay were normalized to the vehicle control and expressed as the % of viability. Next, these data were converted to the Fraction Affected (FA: range 0-1, where FA=0 represents 100% viability, and FA=1 represents 0% viability) and analyzed using the CalcuSyn™ program (Biosoft, Cambridge, UK) based upon the median-effect principle developed by Chou [6]. This program calculates a combination index (CI) that is used to identify synergistic (CI<1), additive (CI=1), and antagonistic (CI>1) flavonoid interactions.

#### *2.5 Cell Cycle Analysis using Flow Cytometry*

The cells were grown in 24-well plates at 37 °C under 5% CO<sub>2</sub> until 80% confluence was reached. The medium was subsequently changed, and flavonoids were added to the indicated concentrations. Next, the cells were incubated at 37 °C for 48 h. After incubation, the cells were harvested and washed three times with ice-cold PBS (pH 7.4). The supernatant was removed, and the cells were washed with 1 mL of PBS and centrifuged at 4 °C. Finally, the supernatant was removed, and 200 µL of 70% ice-cold ethanol and 200 µL of PBS was added to the cells and stored at -20 °C until further use. For use in the flow cytometry experiments, the cell pellet was washed two more times with PBS. The cell pellet was suspended in 0.5 mL of staining reagent (50 µg/mL PI, 50 U/mL RNase, 0.1 mM EDTA, 0.1% Triton X-100, and PBS) and incubated for 30 min at 37 °C in the dark. The DNA fluorescence was measured using a Becton Dickinson (Franklin Lakes, USA) FACScanto II flow cytometer with an excitation wavelength of 488 nm and emission wavelength of 585 nm. Pulse width area signals were used to discriminate between G2 cells and cell doublets. The data were analyzed using FACSDiva Software (Beckton Dickinson). The relative distribution of 104 events for each sample was analysed for background aggregates and debris, an indicator of apoptosis and the G0/G1-, S-, and G2/M-phases of the cell cycle. The control treatments consisted of a culture medium supplemented with FBS. Serum-deprivation treatment was used as an inducer of G0/G1 cell cycle arrest.

#### *2.6 Determination of DNA Synthesis*

DNA synthesis was determined by the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into the DNA making use of a cell proliferation ELISA kit obtained from Roche (Basel, Switzerland). BrdU (10 mM) was added to the medium for the last 2 h of the 48 h treatment with flavonoids (alone or in combination) in a medium supplemented with FBS. Next, the cells were washed twice with PBS and fixed, and the BrdU uptake was determined. The positive growth control consisted of the culture medium supplemented with FBS (complete medium) and the negative control consisted of the medium without FBS. The absorbance was measured using a Multiskan Spectrum microplate reader at 370 nm with a reference wavelength of 492 nm.

#### *2.7 Statistical Analysis*

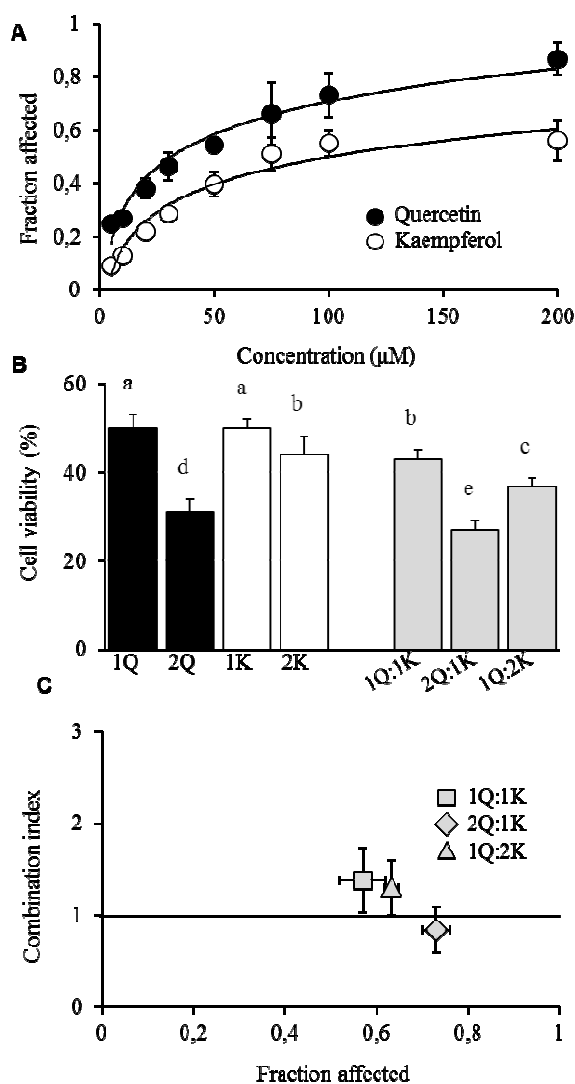
All results are presented as the mean ± SD from triplicate experiments performed in a parallel manner, unless otherwise indicated. Differences in the effects on HCT-116 cells and the different treatments were assessed using ANOVA followed by Duncan's multiple comparison tests using the Statgraphics Plus Program version 2.1. The level of significance used was  $p < 0.05$ .

### 3. Results and Discussion

Quercetin and kaempferol represent 70% of total flavonoid intake [7]. The rationale behind the study of the cytotoxic effect of quercetin in combination with kaempferol on HCT-116 cells has been strengthened by the fact that certain green vegetables and fruits may provide similar amount of both flavonoids (i.e., caper and peach) [8], twice the amount of quercetin than of kaempferol (i.e., tea) [9] or twice the amount of kaempferol than of quercetin (i.e., broccoli and chives) [10]. The question remains of whether quercetin and kaempferol in concert and at different molar ratios could have notably beneficial effects toward achieving cytotoxicity in colon cancer cells.

#### 3.1. Cell Viability

Using the HCT-116 human colon cancer cell line, we first evaluated the effect of flavonoid treatment on cell proliferation by two independent methods: cellular viability assessment-MTT colorimetric assay and cell density measurement-Trypan blue exclusion method. A treatment period of 48 h was selected because the control cells were still in the exponential growth phase at that time. The cell FA was determined after the exposure of cells to quercetin or kaempferol alone (Figure 2A). Both flavonoids were cytotoxic to HCT-116 cells in a time- and dose-dependent manner. The maximal cell death for 48 h exposure was obtained with 200  $\mu$ M quercetin with a percentage of dead cells of nearly 90%, while for kaempferol, the value was 65% for the same concentration. The IC<sub>50</sub> values (the flavonoid concentration required to reduce the initial cell number by 50%) were 40  $\mu$ M for quercetin and 75  $\mu$ M for kaempferol. The concentrations of flavonoids for experiments as single compounds or a combination of compounds were chosen based on the use of IC<sub>50</sub> of the quercetin (1Q) and kaempferol (1K), 2Q (80  $\mu$ M for quercetin), 2K (150  $\mu$ M for kaempferol), and dose ratios of 1Q:1K, 2Q:1K, and 1Q:2K. The interaction of quercetin and kaempferol at a 1Q:1K ratio for 48 h had a slightly greater but still significant effect on HCT-116 cell death than quercetin or kaempferol alone (Figure 2B). However, when cells were exposed at a 2Q:1K ratio, cell death increased acutely, which was an effect that was significantly lower than that obtained with 2Q. When the cells were incubated with a combination of the flavonoids in a 1Q:2K ratio, a significant increase in cell death was caused compared to the flavonoids alone, but it was lower than the result of treatment with the ratio of 2Q:1K. Similar findings were observed for cell density and cell viability measurements using Trypan blue exclusion (data not shown). The dose response data were subsequently evaluated with the CalcuSyn™ program to assess the flavonoid-flavonoid interaction. This procedure estimates the CI values for each flavonoid combination based on the results expected from each of the individual flavonoids. CI values slightly greater than 1 were obtained for the quercetin and kaempferol combination at 1Q:1K and 1Q:2K ratios (Figure 2C), while a CI value slightly lower than 1 was obtained from the 2Q:1K ratio.



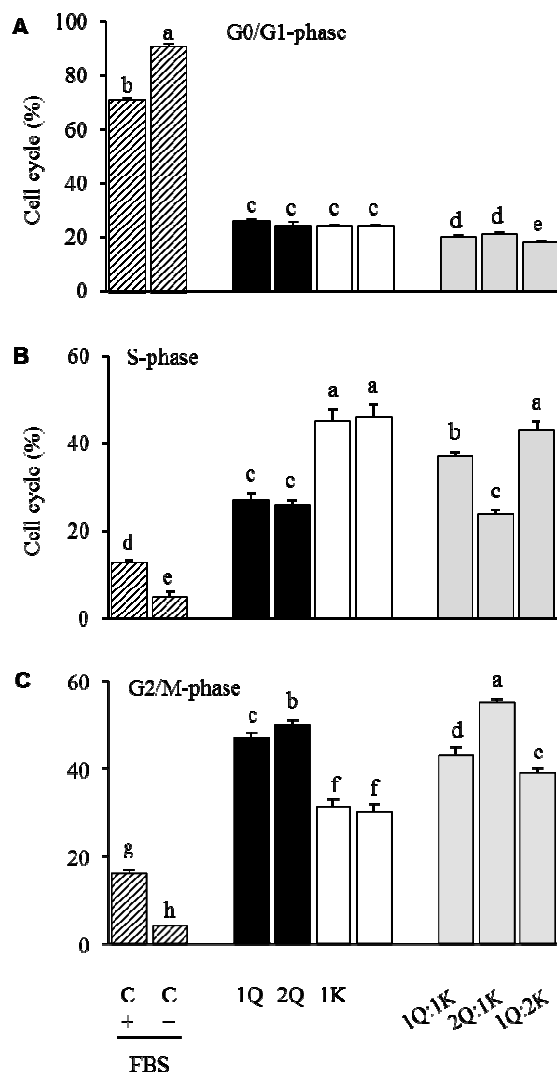
**Figure 2.** Sensitivity of HCT-116 human colon cancer cells to quercetin, kaempferol, and the combination of quercetin and kaempferol. (A) Cells were treated for 48 h with quercetin or kaempferol (0-200  $\mu$ M). Cell viability curves were plotted and reported as the fraction of cells affected by the treatment, where 1 is equivalent to 100% cytotoxicity. (B) Cells were treated for 48 h with IC<sub>50</sub> of the quercetin (1Q), 2Q (80  $\mu$ M), IC<sub>50</sub> of the kaempferol (1K), 2K (150  $\mu$ M), or a dose ratio of 1Q:1K, 2Q:1K, and 1Q:2K in the presence of 10% FBS. (C) The CI was estimated by the CalcuSyn™ software of cell viability curves from cells treated with a dose ratio of 1Q:1K, 2Q:1K, and 1Q:2K and plotted versus the FA. Cell viability was assessed by MTT assay. Each data point or bar represents the mean  $\pm$  SD from 3 replicates. Labeled means without a common letter differ,  $p < 0.05$ .

Our data showed that human colon cancer HCT-116 cells were sensitive to quercetin and kaempferol. According to the IC<sub>50</sub> values determined for the individual flavonoids (1Q and 1K, respectively), quercetin was more potent than kaempferol; however, this cytotoxic efficacy improved significantly when the flavonoids were used in combination in the ratios 1Q:1K, 2Q:1K, and 1Q:2K. It is interesting to notice that quercetin and kaempferol interacted in a synergistic manner on HCT-116 cells only at the 2Q:1K ratio. These findings suggest that the quercetin and kaempferol combination was able to effectively target the cellular machinery crucial to the cell growth of this aggressive, microsatellite-unstable, and growth hormone-independent human colon cancer line [11]. A study conducted by Ackland et al. [12] also revealed that the combination of quercetin and kaempferol is more effective in reducing cell growth in human intestinal lines HuTu-80 and Caco-2 and in the PMC42 breast carcinoma cell line than is either quercetin or kaempferol applied alone. The underlying mechanism of quercetin and kaempferol cytotoxicity on human colon cancer cells is not entirely clear.

In HCT-116 cells, quercetin has been previously shown to trigger apoptosis via NAG-1 under the control of the transcription factors Sp1 and p53 [13], whereas kaempferol activates Ataxia-Telangiectasia Mutated (ATM) and induces apoptosis through the p53-caspase-3 pathway with the involvement of the pro-apoptotic Bcl-2 family members PUMA and Bax [14]. Quercetin and kaempferol have notably similar structures and differ only in the numbers of –OH moieties on the B-ring: two in quercetin and one in kaempferol. In a recent study, Niestroy *et al.* [15] illustrated that the ortho-orientation of the –OH moieties on the B-ring of quercetin, which allows the conversion of the catechol moiety to an ortho-quinone for redox reactions [16], could play a role in the selective inhibition of phase I and phase II detoxification enzymes in human colon cancer cells. The authors hypothesized that ligands presenting structures that are less crowded by –OH moieties incorporate more deeply into the binding site, as a polar pocket in proximity to the heme site of cytochrome P450 [17], and suggested that kaempferol affects the structure of the enzyme in a different manner from quercetin. Other studies also pointed out the flavonoids to selectively accommodate, even with more than one orientation, in binding pockets and induce conformational rearrangements at different catalytic domains of the anti-tumoral target phosphoinositide 3-kinase [18-19]. These observations suggest a reason why colon cancer cells are differently sensitive to these flavonoids and why they show antagonistic interactions at the 1Q:1K and 1Q:2K ratios. Likewise, we do not exclude the possibility that quercetin and kaempferol are mutually exclusive. In fact, other structurally related flavonoids, such as myricetin and naringenin, elicit antagonistic interactions [20]. Our study further suggests a hierarchy of quercetin and kaempferol in the *in vitro* cytotoxic sensitivity of HCT-116 cells. Additional investigations will be necessary to elucidate the synergistic (and antagonistic) mechanism of quercetin and kaempferol, while a fine-tuned combination strategy based on their distinct molecular mechanisms may be warranted.

### 3.2 Cell Cycle

To determine the percentage of HCT-116 cells present in different phases of the cell cycles G0/G1, S, and G2/M, the cells were first synchronized with serum deprivation prior to the treatment with or without quercetin and kaempferol as single compounds (1Q and 1K, respectively) and as a combination in the ratios 1Q:1K, 2Q:1K, and 1Q:2K. Over a 48-h period, serum-deprived cells were progressively accumulated in the G0/G1-phase, whereas serum-supplied cells were present in the S- and G2/M-phases (Figure 3). Treatment with 1Q or 2Q significantly increased the percentage of cells in the G2/M-phase and in the S-phase thereafter. Notably, the percentage of cells in the G2/M-phase increased 4-fold compared to the control after 2Q treatment. Cells exposed to 1K or 2K had a similar cell cycle profile, which was characterized by an increase in cells in the S-phase and subsequently in the G2/M-phase. The combination of flavonoids in the 1Q:1K or 1Q:2K ratios provoked a redistribution in the S- and G2/M phases that fell between the effects of quercetin and kaempferol alone. When the combination of flavonoids was 2Q:1K, the cell cycle profile appeared to mirror that of 1Q or 2Q treatment, but 2Q:1K induced the largest increase in the G2/M-phase.



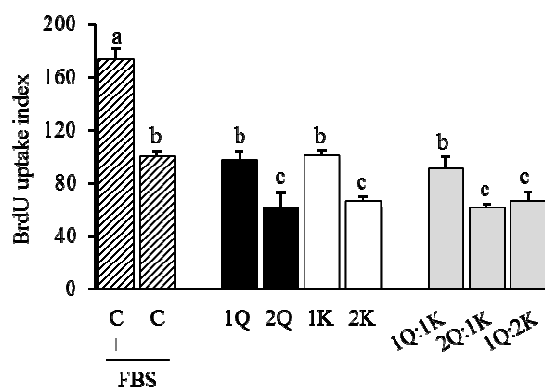
**Figure 3.** Cell cycle profile of HCT-116 human colon cancer cells treated with quercetin, kaempferol, and the combination of quercetin and kaempferol. Cells were treated for 48 h with IC<sub>50</sub> of the quercetin (1Q), 2Q (80  $\mu$ M), IC<sub>50</sub> of the kaempferol (1K), 2K (150  $\mu$ M), or a dose ratio of 1Q:1K, 2Q:1K, and 1Q:2K in the presence of 10% FBS. Flow cytometric analysis was performed to determine the proportion of cells in the G0/G1- (A), S- (B), and G2/M- (C) phases. Each data bar represents the mean  $\pm$  SD from 3 replicates. Labeled means without a common letter differ,  $p < 0.05$ .

These data suggest that quercetin- and kaempferol-induced cytotoxicity in HCT-116 cells is likely to involve the modulation of the cell cycle progression by blocking the S-G0/G1 transition. Interestingly, the population of cells at G2/M was highest when the HCT-116 cells were treated with quercetin and kaempferol in the ratio 2Q:1K, demonstrating that HCT-116 cells arrested at G2/M by a customized combination of quercetin and kaempferol are highly prone to death. Isorhamnetin, an intermediate 3'-ortho-methylated metabolite of quercetin, also promoted G2/M arrest and cytotoxicity in HCT-116 cells, supporting the notion that G2/M checkpoints could be a conserved target for flavonoids in human colon cancer cells [21]. The negative regulation of the complex cyclin B/cdk1 and other positive regulators of cell cycle progression could impair CDK activities and contribute to the quercetin- and kaempferol-induced suppression of the G2/M transition in HCT-116 cells [22]. The cytotoxic potency of isorhamnetin (IC<sub>50</sub> 72  $\mu$ M) was comparable to that of kaempferol (IC<sub>50</sub> 75  $\mu$ M) and inferior to that of quercetin (IC<sub>50</sub> 40  $\mu$ M) in the present study. Therefore, it is possible to tentatively speculate that while the 3'-OH moiety on the B-ring is an important target for phase II metabolism [23] and the antioxidant properties [24] of certain flavonoids, the 3'- and 4'-OH moieties

could also be particularly relevant for flavonoid-dependent defective cell cycle progression and cytotoxicity in HCT-116 cells. Consistent with this argument, Wang *et al.* [25] showed that quercetin and luteolin (i.e., flavonoids having ortho-OH moieties at 3' and 4' positions on the B-ring) exhibited a greater impact on G2/M cell cycle arrest and loss of cell viability than did apigenin, naringenin, chrysin, and acacetin in human SW480 colonic carcinoma cells. Similar observations have been reported recently for other 3',4'-hydroxylated flavonoids (myricetin and laricitrin) in Caco-2 cells [26]. Further studies are necessary to confirm this possibility and elucidate the role of other -OH moieties, derivatives, or double bonds in the phenylchromone structure of flavonoids in affecting the cytotoxic efficacy and mechanistic pathways in the developmental stages of human colon cancer cells.

### 3.3 BrdU Incorporation

To elucidate whether quercetin- and kaempferol-related antiproliferative effects contributed to the reduction of overall cellular viability, we performed a well-established proliferation assay that measured the incorporation of BrdU, a thymidine analogue, into newly synthesized DNA strands of actively proliferating cells during the S-phase. Serum-deprived cells and those treated with 1Q or 1K alone or in combination at a 1Q:1K ratio significantly reduced BrdU incorporation by up to 40% compared to cells incubated in the presence of 10% FBS for 48 h (Figure 4). Cells exposed to 2Q, 2K, 2Q:1K or 1Q:2K displayed a further reduction (65%) in their capacity to accumulate BrdU. The potential anticancer effects of quercetin and kaempferol against HCT-116 cells might result, at least in part, from the inhibition of proliferation verified by its ability to reduce BrdU incorporation. This result was irrespective of the cell cycle in which the cells were arrested, suggesting a flavonoid-induced defect in cells performing DNA repair synthesis with no net DNA synthesis [27].



**Figure 4.** Proliferation of HCT-116 human colon cancer cells treated with quercetin, kaempferol, and the combination of quercetin and kaempferol determined by the incorporation of BrdU into the cells' replicating DNA. Each data bar represents the mean  $\pm$  SD from 3 replicates. Labeled means without a common letter differ,  $p < 0.05$ .

## 4. Conclusions

In summary, we demonstrated the enhanced chemopreventive efficacy of quercetin when associated with kaempferol via the arrest of HCT-116 cells in the G2/M cell cycle phase and an inhibition of DNA synthesis that culminated in the loss of cell viability. These effects support a structure-activity relationship based on the presence of 3'-OH moiety and/or 4'-OH moiety on the B-ring of flavonoids and suggest that eating a customized range of green vegetables and fruits could offer enhanced protection against colon cancer.



## Acknowledgments

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness (AGL2011-29632) and the Junta de Andalucía (P09-CVI-5007). S. Jaramillo-Carmona and S. Lopez acknowledge financial support from Spanish CSIC (JAE-doc Program), contracts co-funded by the ESF.

## Abbreviations

ATM, Ataxia-Telangiectasia Mutated; BrdU, 5-bromo-2'-deoxyuridine; CI, combination index; DMSO, dimethyl sulfoxide; FA, fraction affected, IC50, inhibitory concentration; PI, propidium iodide; FBS, fetal bovine serum.

## References

- [1] S. Tyrovolas and D. B. Panagiotakos (2010). The role of Mediterranean type of diet on the development of cancer and cardiovascular disease, in the elderly: a systematic review, *Maturitas* 65, 122–130.
- [2] J. M. Geleijnse and P. Ch. Hollman (2008). Flavonoids and cardiovascular health: which compounds, what mechanisms?, *Am. J. Clin. Nutr.* 88, 12–13.
- [3] J. M. Calderon-Montaña, E. Burgos-Moron, C. Perez-Guerrero and M. Lopez-Lazaro (2011). A review on the dietary flavonoid kaempferol, *Mini Rev. Med. Chem.* 11, 298–344.
- [4] T. Samuel, K. Fadlalla, L. Mosley, V. Katkoori, T. Turner and U. Manne (2012). Dual-mode interaction between quercetin and DNA-damaging drugs in cancer cells, *Anticancer Res.* 32, 61–71.
- [5] F. J. Duijnhoven, H. B. Bueno-de Mesquita, P. Ferrari, M. Jenab, H. C. Boshuizen, M. M. Ros, C. Casagrande, A. Tjønneland, A. Olsen, K. Overvad, et al. (2009). Fruit, vegetables, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition, *Am. J. Clin. Nutr.* 89, 1441–1452.
- [6] T. C. Chou (2006). Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies, *Pharmacol. Rev.* 58, 621–681.
- [7] Y. Zhang, Y. Li, C. Cao, J. Cao, W. Chen, Y. Zhang, C. Wang, J. Wang, X. Zhang and X. Zhao (2010). Dietary flavonoid and flavone intakes and their major food sources in Chinese adults, *Nutr. Cancer* 62, 1120–1127.
- [8] A. Luximon-Ramma, T. Bahorun, A. Crozier, V. Zbarsky, K. P. Datla, D. T. Dexter and O. I. Aruoma (2005). Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian black teas, *Food Res. Int.* 38, 357–367.
- [9] U.S. Department of Agriculture. USDA Database for the flavonoid content of selected foods [Online] <http://www.nal.usda.gov/fnic/foodcomp/data/flav/flav.html> (accessed March 25, 2003) (2003).
- [10] E. Koh, K. M. S. Wimalasiri, A. W. Chassy and A. E. Mitchell (2009). Content of ascorbic acid, quercetin, kaempferol and total phenolics in commercial broccoli, *J. Food Compos. Anal.* 22, 637–643.
- [11] R. A. Awwad, N. Sergina, H. Yang, B. Ziober, J. K. Willson, E. Zborowska, L. E. Humphrey, R. Fan, T. C. Ko, M. G. Brattain, et al. (2003). The role of transforming growth factor alpha in determining growth factor independence, *Cancer Res.* 63, 4731–4738.
- [12] M. L. Ackland, S. van de Waarsenburg and R. Jones (2005). Synergistic antiproliferative action of the flavonoids quercetin and kaempferol in cultured human cancer cell lines, *In Vivo* 19, 69–76.
- [13] J. H. Lim, J. W. Park, D. S. Min, J. S. Chang, Y. H. Lee, Y. B. Park, K. S. Choi and T. K. Kwon (2007). NAG-1 up-regulation mediated by EGR-1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells, *Apoptosis* 12, 411–421.
- [14] W. Li, B. Du, T. Wang, S. Wang and J. Zhang (2009). Kaempferol induces apoptosis in human HCT116 colon cancer cells via the Ataxia-Telangiectasia Mutated-p53 pathway with the involvement of p53 upregulated modulator of apoptosis, *Chem. Biol. Interac.* 177, 121–127.
- [15] J. Niestroy, A. Barbara, K. Herbst, S. Rode, M. van Liempt and P. H. Roos (2011). Single and concerted effects of benzo[a]pyrene and flavonoids on the AhR and Nrf2-pathway in the human colon carcinoma cell line Caco-2, *Toxicol. In Vitro* 25, 671–683.
- [16] I. M. Rietjens, M. G. Boersma, H. van der Woude, S. M. Jeurissen, M. E. Schutte and G. Alink (2005). Flavonoids and alkenylbenzenes: mechanisms of mutagenic action and carcinogenic risk, *Mutat. Res.* 574, 124–138.
- [17] F. Iori, R. da Fonseca, M. J. Ramos and M. C. Menziani (2005). Theoretical quantitative structure–activity relationships of flavone ligands interacting with cytochrome P450 1A1 and 1A2 isozymes, *Bioorg. Med. Chem.* 13, 4366–4374.
- [18] E. H. Walker, M. E. Pacold, O. Perisic, L. Stephens, P. T. Hawkins, M. P. Wymann and R. L. Williams (2000). Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine, *Mol. Cell* 6, 909–919.

- [19] K. Almhanna, J. Strosberg and M. Malafa (2011). Targeting AKT protein kinase in gastric cancer, *Anticancer Res.* 31, 4387–4392.
- [20] B. L. Freeman, D. L. Eggett and T. L. Parker (2010). Synergistic and antagonistic interactions of phenolic compounds found in navel oranges, *J. Food Sci.* 75, 570–576.
- [21] S. Jaramillo, S. Lopez, L. M. Varela, R. Rodriguez-Arcos, A. Jimenez, R. Abia, R. Guillen and F.J. Muriana (2010). The flavonoid isorhamnetin exhibits cytotoxic effects on human colon cancer cells, *J. Agric. Food Chem.* 58, 10869–10875.
- [22] A. Lindqvist, V. Rodriguez-Bravo and R. H. Medema (2009). The decision to enter mitosis: feedback and redundancy in the mitotic entry network, *J. Cell Biol.* 185, 193–202.
- [23] H. Van der Woude, M. G. Boersma, J. Vervoort and I. M. C. M. Rietjens (2004). Identification of 14 quercetin phase II mono- and mixed conjugates and their formation by rat and human phase II in vitro model systems, *Chem. Res. Toxicol.* 17, 1520–1530.
- [24] P. Trouillas, P. Marsal, D. Siri, R. Lazzaroni and J. L. Duroux (2006). A DFT study of the reactivity of OH groups in quercetin and taxifolin antioxidants: The specificity of the 3–OH site A, *Food Chem.* 97, 679–688.
- [25] W. Wang, P. C. Van Alstyne, K. A. Irons, S. Chen, J. W. Stewart and D. F. Birt (2004). Individual and interactive effects of apigenin analogs on G2/M cell-cycle arrest in human colon carcinoma cell lines, *Nutr. Cancer* 48, 106–114.
- [26] S. Gomez-Alonso, V. J. Collins, D. Vauzour, A. Rodriguez-Mateos, G. Corona and J. P. E. Spencer (2012). Inhibition of colon adenocarcinoma cell proliferation by flavonols is linked to a G2/M cell cycle block and reduction in cyclin D1 expression, *Food Chem.* 130, 493–500.
- [27] G. D. Kao, W. G. McKenna and T. J. Yeng (2001). Detection of repair activity during the DNA damage-induced G2 delay in human cancer cells, *Oncogene* 20, 3486–3496.

**A C G**  
**publications**

© 2014 ACG Publications