Quercetin Inhibits Human DLD-1 Colon Cancer Cell Growth and Polyamine Biosynthesis

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Abstract. *Background/Aim: Polyamines and ornithine decarboxylase are involved in cell growth and differentiation. The polyphenol quercetin may exert anti-tumour properties by influencing proliferation, differentiation, and apoptosis. The aim of the study was to investigate the effects of increasing concentrations of quercetin (from 0.1 to 100 μM) on polyamine biosynthesis, cell proliferation, and apoptosis in the DLD-1 cells. Materials and Methods: Polyamine levels and ornithine decarboxylase activity were evaluated by HPLC and radiometric technique, respectively. The proliferative response was estimated by 3-(4,5 di-methylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide (MTT) test and [^{3}H]-thymidine incorporation in cell DNA. Apoptosis was investigated by DNA fragmentation. Results: At concentrations ≥50 μM, quercetin significantly reduced ornithine decarboxylase activity, putrescine and spermidine levels compared to controls and cells treated with 0.1 μM concentration. Quercetin concentrations ≥70 μM caused a significant reduction in the conversion of MTT tetrazolium salt and [^{3}H]-thymidine incorporation. The same concentrations were needed to induce the apoptosis. Conclusion: The present study demonstrates that quercetin can affect growth of DLD-1 cells by both decreasing polyamine biosynthesis and inducing apoptosis. Due to the extensive dietary consumption of polyphenols, such as quercetin, the biological activity of these compounds deserves further investigation.*

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The flavonoids are the largest class of polyphenolic compounds widely distributed in legumes, fruits and vegetables, as well as in beverages such as tea and red wine. As components of edible plants and plant foodstuffs, they constitute an integral part of the human diet (1, 2). Among flavonoids, quercetin is the most important, being particularly abundant in onions, apples, tea, and broccoli, with an estimated daily intake of 25-30 mg in Europe (3). A number of physiological benefits have been attributed to flavonoid consumption, including protection from cardiovascular disease and cancer, but the precise mechanisms underlying these activities remain to be determined (4-6).

In this framework, it has been observed that flavonoids exhibit antitumour properties, likely due to immune stimulation, free radical scavenging, alteration of the mitotic cycle in tumour cells, gene expression modification, anti-angiogenesis activity, apoptosis induction, or, more probably, a combination of these effects (7, 8). *In vitro* experiments have demonstrated that among flavonoids, quercetin inhibits cell proliferation and induces apoptosis in many types of cancer cells through different signalling pathways (9-12). Recent studies in animal models have confirmed that quercetin is also able to inhibit tumour development (13-15). Evidence indicates how quercetin seems mainly to restrain neoplastic cell growth by inhibiting various tyrosine kinases (6), even if other mechanisms, as hypothesized for other compounds, might also contribute to the net effect.

In our previous study (16), we reported that the isoflavone genistein, a major component of soy, can affect growth of DLD-1 human colon cancer cells by both inducing apoptosis and decreasing polyamine biosynthesis. These naturally occurring polycationic compounds, namely putrescine, spermidine, and spermine, are ubiquitous short-chain aliphatic amines that have been detected in all eukaryotic cells studied and demonstrate an active role in cell proliferation and differentiation. Abnormal hyperproliferative cells (*e.g.* those in neoplastic and preneoplastic tissue) exhibit very high requirements for polyamines to sustain cell growth through elevated DNA, RNA, and protein synthesis (17).

Cellular polyamine homeostasis is maintained through the concerted effort of feedback systems controlling polyamine transport as well as specific key enzymes in polyamine metabolism, such as ornithine decarboxylase (ODC). ODC is a rate-limiting enzyme that is highly regulated in all cells and responds to a wide variety of growth-promoting stimuli (18). Animal models have also demonstrated that in the rat mammary epithelium, ODC activity and polyamine concentrations were significantly lower in the group treated with soy protein than the control (19).

The association between genistein-induced modulation of polyamines metabolism with cellular proliferation and apoptosis in colon cancer cells, provided a new mechanism by which polyphenols may exert their anti-neoplastic effect. Therefore, depending on the biological properties shown by substances such as genistein, it is interesting to evaluate the putative proapoptotic and anti-proliferative effects of other compounds such as the flavonol quercetin in relation to polyamine metabolism, keeping in mind that these two compounds are normal components of two different dietary regimens: Asian populations consume a diet that is very rich in the phytoestrogen genistein, whereas a traditional Mediterranean diet is abundant in quercetin deriving from vegetables, fruits, nuts and legumes/beans. In the present study, we investigated whether increasing concentrations of quercetin are able to affect the polyamine biosynthesis in the DLD-1 human colon cancer cell line. In addition, we also investigated its effects on cell growth and apoptosis on the same cell line.

Materials and Methods

Cell culture. Human colon adenocarcinoma-derived DLD-1 cells were obtained from the Interlab Cell Line Collection (IST, Genoa, Italy). Cells were routinely cultured in RPMI-1640 supplemented with 10% foetal bovine serum (FBS), 1% nonessential amino acids, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, in a monolayer culture, and incubated at 37˚C in a humidified atmosphere containing 5% CO_2 in air. At confluence, the grown cells were harvested by means of trypsinization and serially subcultured with a 1:4 split ratio. All cell culture components were purchased from Sigma-Aldrich (Milan, Italy).

Quercetin treatment. In the experiments investigating the effects of quercetin, DLD-1 cells were seeded at a density of 2×10^5 cells/5ml of RPMI 1640 containing 10% fetal bovine serum (FBS) in 60 mm tissue culture dishes (Corning Costar Co., Milan, Italy). The cells were incubated for 24 hours, to allow for attachment, and then the medium was replaced by fresh culture medium containing quercetin at increasing concentrations (0.1 μM, 0.5 μM, 1 μM, 10 μM, 20 μM, 50 μM, 70 μM, and 100 μM) dissolved in dimethyl sulfoxide (DMSO). In these experimental conditions, the DLD-1 cells were allowed to grow for 24 hours and 48 hours and then processed for subsequent analyses. Each experiment included an untreated control and a control with the equivalent concentration of DMSO as the one used for adding quercetin. Triplicate cultures were set up for each flavonoid concentration and for the control, and each experiment was repeated 4 times. Cell viability, determined using the trypan blue exclusion test, always exceeded 90%.

Polyamine analysis. For the evaluation of polyamine levels after quercetin treatment, each cell culture pellet was homogenized in 700 μl of 0.9% sodium chloride mixed with 5 μl (174 nmol/ml) of an internal standard (1,10-Diaminodecane). To precipitate the protein, 50 μl of perchloric acid 3M were added to the homogenate. After 30 min of incubation in ice, the homogenate was centrifuged for 15 min at 7,000×*g*. The supernatant was filtered (Millex-HV13 pore size 0.45 mm; Millipore, Bedford, MA) and lyophilised. Aliquots (100 μl) were reacted with dansyl chloride, and the dansyl-polyamine derivatives were determined by high-performance liquid chromatography as previously described (20). Polyamine levels were expressed as concentration values in nm/mg of protein.

ODC activity. ODC activity was measured with a radiometric technique that estimated the amount of $^{14}CO_2$ liberated from DL-[1-^{14}C]- ornithine (specific activity, 56.0 mCi/mml; New England Nuclear, Monza, Italy) (20). The cell culture pellet ($2\text{-}4\times10^6$ cells) was homogenized in 0.6 ml ice-cold Tris-HCl (15 mM, pH 7.5) containing 2.5 mM dithiothreitol, 40 μM pyridoxal-5'-phosphate, and 100 μM ethylene diamine tetra acetate and then centrifuged at 30,000×*g* for 30 min at 4˚C.

An aliquot of supernatant (200 μl) was added to a glass test tube containing 0.05 μCi DL-[1-^{14}C]-ornithine and 39 nmol DL-ornithine. After incubation for 60 min at 37˚C, the reaction was stopped by adding TCA to a final concentration of 50%. $^{14}CO_2$ liberated from DL-[1-^{14}C]- ornithine was trapped on filter paper pre-treated with 40 μl NaOH (2 N), which was suspended in a central well above the reaction mixture. Radioactivity on the filter papers was determined by a liquid scintillation counter (Model 1219 Rackbeta; LKB-Pharmacia, Uppsala, Sweden). ODC activity was expressed as pmol CO_2/h/mg of protein. Enzymatic activity was found to be linear within the range of 50-600 μg of protein (r^2=0.99). The intra-assay and inter-assay of coefficients variation (CV%) were 6% and 8%, respectively.

Assessment of cell proliferation. After DLD-1 cells had been cultured for 24 hours and 48 hours with increasing concentrations of quercetin, the proliferative response was estimated by the colorimetric 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test and the [^{3}H]-thymidine incorporation in cellular DNA.

To determine cell growth by the colorimetric test, MTT stock solution (5 mg/ml in medium) was added to each dish at a volume of one-tenth the original culture volume and incubated for 2 hours at 37˚C in humidified CO_2. At the end of the incubation period, the medium was removed, and the blue formazan crystals were solubilized with acidic isopropanol (0.1 N HCl in absolute isopropanol). MTT conversion to formazan by metabolically viable cells was monitored using a spectrophotometer at an optical density of 570 nm.

To determine DNA synthesis, 0.3 μCi/ml of [methyl-^{3}H]-thymidine (85.50 Ci/mmol; NEN Life Science Products Inc., Boston, MA) was added to triplicate dishes in the last 12 hours of quercetin treatment. After incubation, the medium was aspired to remove unincorporated [^{3}H]-thymidine, and the cells were maintained with 0.33 N NaOH for 30 min. To precipitate and hydrolyze the DNA, the resulting cells were harvested by collection onto glass tubes containing 40% TCA with 1.2 N HCl and centrifuged at 3,000×*g* for 15 min. The precipitated DNA was re-

Table I. *The effects of increasing concentrations of quercetin on the polyamine content of DLD-1 cells.*

24 h	Control	0.1 μM	0.5 μM	1 μM	10 μM	20 μM	50 μM	70 μM	100 μM
Putrescine	0.09[a] (0.06-0.12)	0.09[ac] (0.06-0.12	0.06[ab] (0.06-0.09)	0.04[ab] (0.01-0.07)	0.04[ab] (0.03-0.06)	0.04[ab] (0.03-0.05)	0.04[bc] (0.03-0.05)	0.04[bc] (0.03-0.05)	0.03[b] (0.03-0.04)
Spermidine	4.35[a] (2.3-5.7)	3.75[a] (2.2-5.7)	2.77[ab] (1.9-3.0)	2.17[ab] (1.5-2.8)	1.95[ab] (1.8-3.3)	1.80[ab] (1.5-2.5)	1.75[b] (1.5-1.8)	1.75[b] (1.5-1.8)	1.77[b] (1.7-1.8)
Spermine	9.30 (7.1-13.8)	8.92 (6.7-13.5)	7.95 (5.5-10.3)	8.25 (6.0-9.0)	8.55 (7.2-9.0)	8.17 (6.4-8.8)	8.55 (6.3-9.0)	7.74 (6.6-0.89)	6.01 (5.1-7.8)
Total polyamines	13.74[a] (9.4-19.6)	12.76[a] (9.1-18.7)	10.80[a] (7.6-13.4)	10.48[a] (8.0-11.6)	10.50[a] (9.0-2.3)	10.16[a] (8.05-10.8)	10.16[a] (8.0-10.8)	9.34[b] (8.4-12.8)	7.83[b] (6.9-9.5)
48 h	Control	0.1 μM	0.5 μM	1 μM	10 μM	20 μM	50 μM	70 μM	100 μM
Putrescine	0.30[a] (0.26-0.32)	0.29[ac] (0.26-0.32)	0.26[ab] (0.26-0.29)	0.25[ab] (0.215-0.27)	0.25[ab] (0.23-0.26)	0.25[ab] (0.23-0.25)	0.25[bc] (0.23-0.25)	0.25[bc] (0.23-0.25)	0.24[b] (0.23-0.24)
Spermidine	5.55[a] (3.4-6.9)	4.95[a] (3.4-6.9)	3.97[ab] (3.1-4.2)	3.37[ab] (2.7-4.0)	3.15[ab] (3.0-4.5)	3.00[ab] (2.7-3.7)	2.95[b] (2.7-3.1)	2.95[b] (2.7-3.0)	2.97[b] (2.9-3.1)
Spermine	11.30 (9.1-15.8)	10.93 (8.7-15.5)	9.95 (7.5-12.3)	10.25 (8.0-11.0)	10.55 (9.2-11.0)	10.18 (8.4-10.8)	10.55 (8.3-11.0)	9.74 (8.6-12.9)	8.01 (7.1-9.8)
Total polyamines	17.14[a] (12.8-23.0)	16.16[a] (12.49-22.12)	14.20[a] (10.9-16.8)	13.88[a] (11.4-14.9)	13.90[a] (12.4-15.7)	13.65[a] (11.5-14.3)	13.50[a] (11.4-14.2)	12.75[b] (11.8-16.2)	11.23[b] (10.3-12.9)

For each time period (24 hours and 48 hours) and quercetin concentration, median values not sharing a common superscript (a, b, and c) differ significantly ($P< 0.05$, Kruskal–Wallis analysis of variance and Dunn's multiple comparison test).

dissolved in 0.33 N NaOH, and then 250 μl were transferred into vials containing 3 ml of scintillation fluid. Incorporation of [^{3}H]-thymidine in DNA was determined by scintillation quantitation in a Rackbeta counter (model 1219; LKB-Pharmacia, Turku, Finland).

Apoptotic death assay. After DLD-1 cells had been cultured for 24 hours and 48 hours with quercetin at concentrations between 0.1 μM and 100 μM, the cytosolic DNA–histone complexes generated during apoptotic DNA fragmentation were evaluated with a cell death detection enzyme-linked immunoabsorbent assay (ELISA) kit (Roche Diagnostics GmbH, Mannheim, Germany) following the supplier's instructions. Each experiment was performed 4 times.

Statistical analysis. Due to the non normal distribution of the data, non parametric tests were performed. For ODC activity, polyamine levels and proliferative characteristics of DLD-1 cells, the significance of differences between the groups was determined by the Kruskal Wallis analysis of variance. Comparisons between each single treatment group were analyzed with the Wilcoxon Mann Whitney test. Correlations between single and total polyamine content and increasing quercetin concentrations were analyzed using the Spearman correlation test. All data are expressed as median value and range. Differences were considered significant at $P<0.05$. A specific software package (SigmaStat for Windows version 3.00 SPSS Inc. San Jose, CA, USA) was used.

Results

Effects of quercetin on polyamine biosynthesis. The effects of quercetin on ODC activity were studied at increasing concentrations (namely 0.1 μM, 0.5 μM, 1 μM, 10 μM, 20 μM, 50 μM, 70 μM, and 100 μM) for 24 hours and 48 hours, respectively. As shown in Figure 1, after 24 hours administration, quercetin concentrations equal to or higher than 50 μM significantly reduced ODC activity compared to that in untreated cells and in cells treated with quercetin concentrations equal to or lower than 1 μM ($P<0.05$). After a 48-hour administration, ODC activity was significantly reduced by quercetin concentrations equal to or higher than 50 μM compared to untreated cells. In addition, 100 μM significantly reduced ODC activity in comparison to quercetin concentrations equal to or lower than 1 μM.

Regarding the polyamine profile, the administration of increasing concentrations of quercetin (from 0.1 μM to 100 μM) after 24 hours and 48 hours led to a decrease of the single and total polyamine contents in the DLD-1 cells (Table I). The decrease was significant ($P<0.05$) with concentrations equal to or higher than 50 μM for putrescine and spermidine compared to untreated control cells and cells treated with 0.1 μM concentration. Additionally, the total polyamine content decreased significantly ($P<0.05$) with concentrations equal to or higher than 70 μM.

Finally, the polyamine content was found to be inversely correlated with quercetin concentrations. The significant and inverse relationship between quercetin concentrations after 24 hours of treatment and the total polyamine content in the DLD-1 cells is reported in Figure 2 (r=–0.8285, P=0.0083. Spearman correlation coefficient). The effect was equivalent after 48 hours of treatment and a significant inverse correlation was also present for the spermidine content at the same times of administration (data not shown).

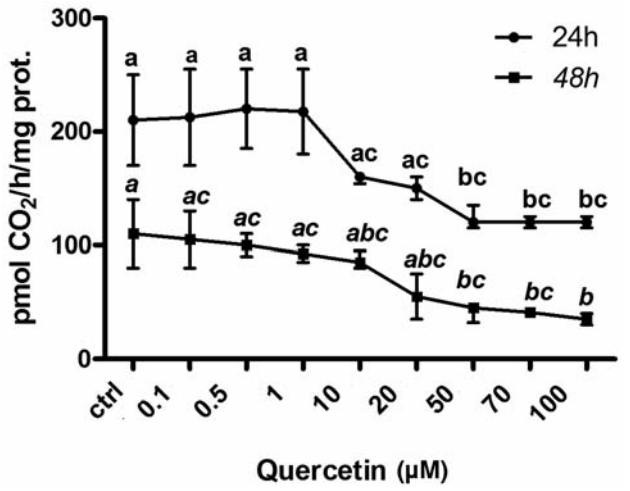
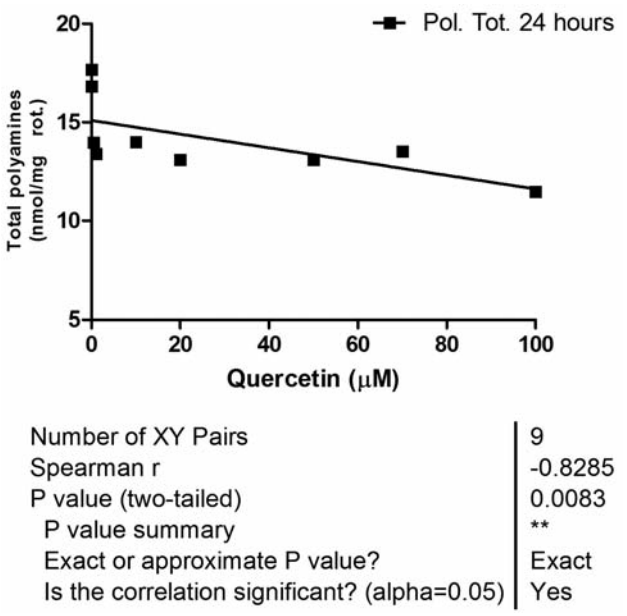


Figure 1. *Effects of increasing concentrations of quercetin on the conversion of MTT tetrazolium salt in DLD-1 cells after 24 hours and 48 hours of treatment, respectively. Quercetin was administered at increasing concentrations (0.1 μM, 0.5 μM, 1 μM, 10 μM, 20 μM, 50 μM, 70 μM, and 100 μM). All data represent the results of four different experiments (median value and the range). For each time (24 hours and 48 hours) and quercetin concentration, median values not sharing a common superscript differ significantly (P<0.05, Kruskal–Wallis analysis of variance and Dunn's multiple comparison test).*



Number of XY Pairs	9
Spearman r	-0.8285
P value (two-tailed)	0.0083
P value summary	**
Exact or approximate P value?	Exact
Is the correlation significant? (alpha=0.05)	Yes

Figure 2. *The significant and inverse relationship between quercetin concentrations and the total polyamine content in the DLD-1 cells after 24 hours' administration (r=–0.8285, P= 0.0083, Spearman correlation coefficient).*

Effects of quercetin on DLD-1 cells proliferation. Figure 3 panel A shows the effects of increasing concentrations of quercetin on the conversion of MTT tetrazolium salt in DLD-1 cells after 24 hours and 48 hours of treatment, respectively. Exposure of the DLD-1 cell line to increasing concentrations of quercetin (from 0.1 μM to 100 μM) caused an evident anti-proliferative effect. After 24 hours, concentrations equal to or higher than 70 μM caused a significant reduction in the conversion of the MTT tetrazolium salt compared to untreated control cells and cells treated with concentrations equal to or lower than 1 μM ($P<0.05$). After 48 hours of treatment, the reduction was caused by concentrations of quercetin equal to or higher than 70 μM compared to the untreated control cells and cells treated with 0.1 μM ($P<0.05$). Moreover, cells treated with 100 μM concentration showed a significant reduction in conversion of the MTT tetrazolium salt compared with cells treated with concentrations equal to 0.5 μM and 1 μM ($P<0.05$).

The effects of increasing concentrations of quercetin on the incorporation of [^{3}H]-thymidine in the DNA of DLD-1 cells are shown in Figure 3, panel B. After 24 hours, concentrations equal to or higher than 70 μM of quercetin caused a significant reduction in [^{3}H]-thymidine incorporation in DNA of cells compared with the untreated control cells and cells treated with concentrations equal to or lower than 1 μM ($P<0.05$). This observation was similar to that for MTT. Finally, after 48 hours the reduction in the [^{3}H]-thymidine incorporation was significant ($P<0.05$) with quercetin concentrations equal to or higher than 70 μM concentration compared to the untreated control cells and cells treated with 0.1 μM ($P<0.05$).

Effects of quercetin on apoptosis. Figure 4 shows the effects of increasing quercetin concentrations (from 0.1 μM to 100 μM) on the apoptosis of DLD-1 cells, evaluated by cell death ELISA test. After both 24 hours and 48 hours, concentrations of quercetin equal to or higher than 70 μM increased the apoptotic process significantly ($P<0.05$) compared with control cells. Additionally, quercetin at 100 μM concentration induced a significant increase in apoptosis compared with cells treated with 0.1 μM concentration.

Discussion

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin and quercetin is the major representative of the flavonol subclass. Plant products (*e.g.* fruit, vegetables, cereals, leguminous plants, fruit juices) and beverages, such as tea and red wine, constitute the main sources of quercetin. There is substantial evidence to support that flavonoid compounds exert a protective role in tumour development, especially in the intestinal tract due to the direct exposure of intestinal epithelia to these dietary ingredients. However, epidemiological studies relating the

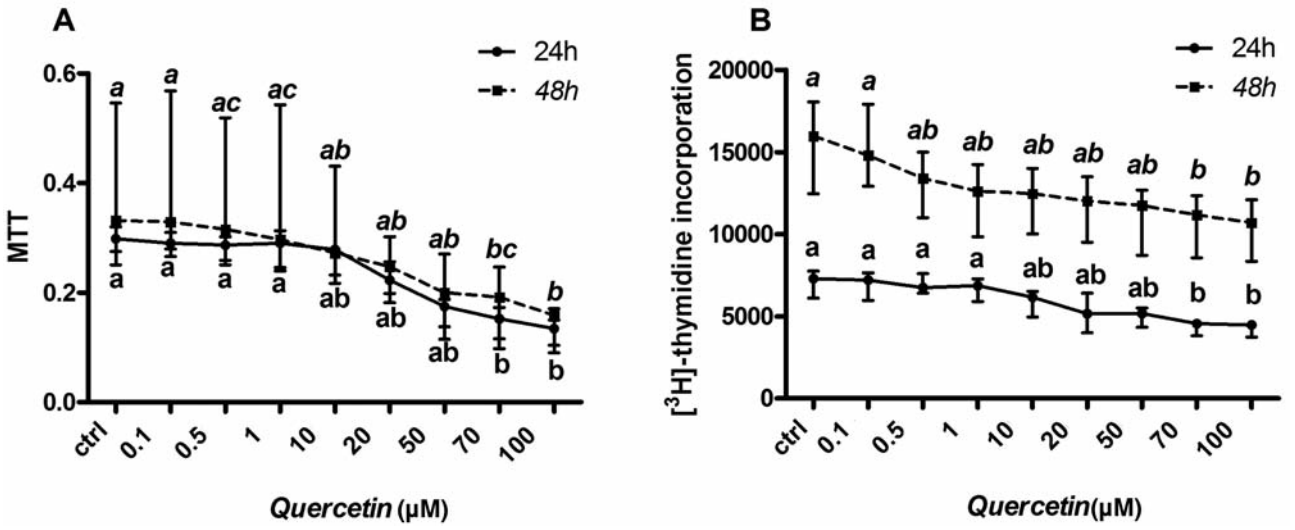


Figure 3. *Effects of increasing concentrations of quercetin on the ODC activity in DLD-1 cells after 24 hours and 48 hours of treatment, respectively. Quercetin was administered at increasing concentrations (0.1 μM, 0.5 μM, 1 μM, 10 μM, 20 μM, 50 μM, 70 μM and 100 μM). All data represent the results of four different experiments (median value and the range). For each time (24 hours and 48 hours) and quercetin concentration, median values not sharing a common superscript differ significantly (P<0.05, Kruskal–Wallis analysis of variance and Dunn's multiple comparison test).*

quercetin intake to risk of colorectal cancer have not produced conclusive data thus far. On the other hand, direct evidence of the protective effect of this flavonol against colorectal carcinogenesis has been reported in experimental colorectal cancer animal model studies (13, 14) as well as *in vitro* experiments (12, 21). These effects include the inhibition of the cancer initiation phase due to its anti-oxidative (22, 23), antimutagenic (24), and antiproliferative properties (12, 26). In addition, quercetin has also been reported to delay cancer progression by inducing apoptosis (12) and inhibiting angiogenesis (26).

Data from the present study demonstrate that administration of increasing quercetin concentrations to DLD-1 cells at both 24 hours and 48 hours of treatment influenced proliferation and apoptosis. Particularly, by employing concentrations between 0.1 μM and 100 μM, DLD-1 cells showed a significant anti-proliferative effect, as demonstrated by the significant reduction in MTT conversion and [^{3}H]-thymidine incorporation, with the highest quercetin concentrations (starting from 70 μM). Moreover, quercetin proved to be effective in inducing cell death by apoptosis, causing a significant increase in the DNA fragmentation rate obtained with the same concentrations.

In spite of the large amount of data published on the presumed ability of quercetin in affecting neoplastic cell growth, the precise mechanisms are not yet elucidated (9, 12). Therefore, further attempts to identify all the actors playing a role in this process are noteworthy.

Among the substances actively involved in cell proliferation and growth, polyamines and their rate-limiting

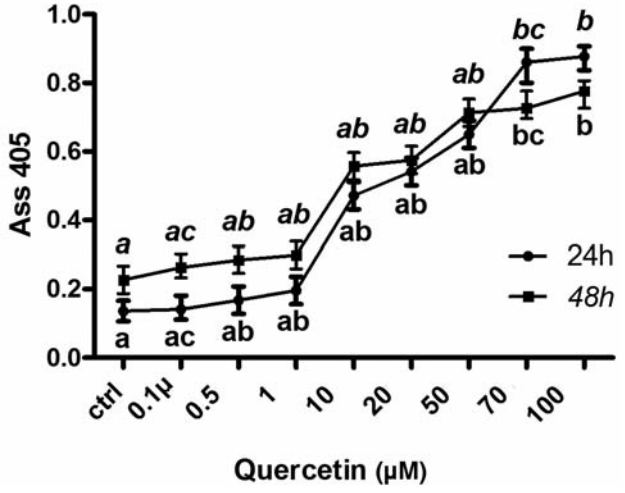


Figure 4. *Effects of increasing concentrations of quercetin (from 0.1 μM to 100 μM) on the apoptosis of DLD-1 cells, evaluated by cell death ELISA test after 24 hours and 48 hours of treatment, respectively. Quercetin was administered at increasing concentrations (0.1 μM, 0.5 μM, 1 μM, 10 μM, 20 μM, 50 μM, 70 μM, and 100 μM). All data represent the results of four different experiments (median value and the range). For each time period (24 hours and 48 hours) and quercetin concentration, median values not sharing a common superscript differ significantly (P<0.05, Kruskal–Wallis analysis of variance and Dunn's multiple comparison test).*

enzyme, ODC, are crucial and it is well known that high expression of ODC along with increased polyamine concentrations are associated with fast proliferating cells (27). In colorectal cancer, polyamine levels are significantly

increased compared with either normal or preneoplastic tissue (20), and are believed to be reliable markers of neoplastic cell proliferation. In this framework, the main result of the present study is that quercetin at concentrations starting from 50 μM induced a significant decrease in ODC activity. At the same concentration, a significant decrease in the putrescine and spermidine, but not for spermine, contents occurred.

The significant reduction of ODC activity in DLD-1 cells by quercetin may account for the observed variations in the single polyamine contents. ODC is a key regulator in polyamine metabolism, being now considered as a true oncogene (28). This enzyme influences mainly putrescine and spermidine levels, which are more involved in cell proliferation than spermine; the latter is implicated essentially in cell differentiation and neoplastic transformation, with different processes involved in maintaining its critical levels (29). Depletion of polyamines can lead to cell cycle arrest or apoptosis by affecting numerous cell cycle regulatory pathways (30). Moreover, polyamines are important in the regulation of ion transport and the stabilization of important cellular components such as cell membranes and chromatin structure. Therefore, a reduction of the levels of these polycations might induce destabilization of important cell structures, leading to loss of cell integrity and finally inducing cell death. Additionally, the correlations observed between polyamine levels and MTT support the hypothesis that polyamines are strongly associated with the ability of quercetin to reduce growth and induce apoptosis of DLD-1 colon cancer cells, indicating that the molecular mechanism underlying the anti-tumour effect of quercetin may involve the suppression of polyamine synthesis *via* ODC control. With regard to the required concentration of quercetin (50 μM) for significantly inhibiting polyamine biosynthesis, it was lower than that required for reducing MTT conversion and [^{3}H]-thymidine incorporation (70 μM). Most likely, exposure to a lower concentration of this flavonol could be sufficient for polyamines, which are necessary for cells to initiate their proliferative processes, since their synthesis is an early event during the G_1 phase of cell cycle (31).

To our knowledge, this is the first study investigating the effects of quercetin administration on polyamine metabolism. Our findings are in agreement with other data in literature about the relationship between polyamine biosynthesis and other polyphenolic substances during carcinogenesis and tumour growth (32). Interestingly, the cancer-preventing activities shown by quercetin in an *in vitro* model such as DLD-1 cells are comparable to those for other flavonoids such as curcumin and genistein. In a previous study by our group, treatment of the same DLD-1 cell line with genistein, the predominant soy isoflavone, led to a similar inhibition of proliferation and enhancement in apoptosis by changes in polyamines biosynthesis, although with concentrations much lower than those used for quercetin (16).

This discrepancy could be attributed to the different bioefficacy shown by the different classes of polyphenols (33). Moreover, it must be highlighted that the *in vitro* concentrations of quercetin required to obtain the antitumour effects described above on DLD-1 colon cancer cells (≥50 μM) far exceed the normal intake of quercetin from a diet rich in flavonol products (34). Thus, to obtain anti-carcinogenetic effects from quercetin, a supra-physiological intervention would be required and this could be the reason for the lack of antineoplastic effects observed in population studies. This could also depend on quercetin bioavailability. Although very abundant in our diet, this substance is poorly absorbed and its action is thus restricted to the intestine. In general, intakes of monomeric flavonols, flavones, and flavanols are relatively low, and plasma concentrations rarely exceed 1 μmol/l due to limited absorption and rapid elimination (34). In this context, further studies in humans are required to support the observed antiproliferative and pro-apoptotic properties, also focusing better on the doses needed to obtain *in vivo* the same relevant effects produced in experimental models. From a therapeutical point of view, more probably, combinations of natural substances such as quercetin with other compounds such as polyamine inhibitors and/or analogues, would enhance their properties, representing a suitable option for chemoprevention and/or treatment of colon cancer.

In conclusion, it is becoming increasingly crucial to gain information on how natural substances present in daily foods could be of help in a strategy of cancer chemoprevention, leading to modifications in lifestyle and food intake. From a mechanistic point of view, this study suggests that quercetin can affect growth of DLD-1 cells by both decreasing polyamine biosynthesis and inducing apoptosis.

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