Antiproliferative effect of quercetin in the human U138MG glioma cell line

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Recent epidemiological and dietary intervention studies in animals and humans have suggested that diet-derived flavonoids, in particular quercetin, may play a beneficial role by preventing or inhibiting tumorigenesis. The aim of this study was to evaluate whether quercetin may act differently on cancer and normal neuronal tissue.
In order to investigate this, the U138MG human glioma cell line and hippocampal organotypic cultures were used. The study showed that quercetin induced in glioma cell cultures results in (a) a decrease in cell proliferation and viability, (b) necrotic and apoptotic cell death, (c) arrest in the G_2 checkpoint of the cell cycle, and (d) a decrease of the mitotic index. Furthermore, we demonstrated that while quercetin promotes cancer regression it was able to protect the hippocampal organotypic cultures from ischemic damage. To sum up, our results suggest that quercetin induced growth inhibition and cell death in the U138MG human glioma cell line, while exerting a cytoprotective effect in normal cell cultures. *Anti-Cancer Drugs* 17:663–671 © 2006 Lippincott Williams & Wilkins.

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Introduction

Malignant gliomas are tumors that arise from cells of astrocytic lineage, and are considered the commonest and most devastating primary tumors in the brain, representing 50–60% of this type of tumor. As a result of high proliferation, invasiveness, and resistance to radiation [1] displayed by malignant gliomas, the prognosis for patients with these tumors is poor and mean survival is less than 12 months [2]. Despite intense efforts to develop treatments, effective agents are still not available.

Recent epidemiological and dietary intervention studies in animals and humans have suggested that diet-derived phenolics, in particular the flavonoids, may play a beneficial role in inhibiting, reversing or retarding tumorigenesis [3]. Antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities were shown for these compounds [4]. Flavonoids can interfere with a large number of mammalian enzymes, such as detoxifying enzymes [5] that are involved in major cell division and proliferation pathways. Among the flavonoids, quercetin (3,3′,4′,5,7-pentahydroksyflavon) is one of the most widespread in the plant kingdom, and occurs naturally in a wide range of fruits and vegetables [6]. Several studies have shown that quercetin holds a broad range of pharmacological properties that include selective antiproliferative effects [7] and cell death, predominantly through an apoptotic mechanism in cancer cell lines but not in normal cells [8]. This antiproliferative effect is exerted by producing arrest in phase G_1 of the cell cycle [9] through interaction with cell cycle-regulated proteins, like cyclin D1 and CDK-4 [10]. Quercetin is thought to induce release of cytochrome *c* and activation of caspase-9 and caspase-3 in HL-60 cells, thereby triggering apoptosis [11]. Moreover, this flavonoid is a potential phosphotidylinositol-3-kinase (PI3K) inhibitor, thus blocking this pivotal cell survival pathway [12]. Therefore, the ability of quercetin to prevent and/or to retard tumor growth is potentially a multitarget effect. The study of which targets are involved in the quercetin effects in glioma cells can be important to evaluate the potential pharmacological use of this flavonoid for this type of tumor.

Thus, considering that (a) flavonoids are generally safe and without adverse effects, many flavonoids occur in our diet and the antiproliferative effects of quercetin appears selectively to tumoral cells, (b) abnormalities in several signaling molecules have been implicated in gliomagenesis and many investigations demonstrated the potential

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interactions between quercetin and cell proliferation pathways, and, finally, (c) studies *in vitro* demonstrated that flavonoids are able to traverse the blood–brain barrier [13], we further examined the quercetin effects on proliferation, cell cycle and selective cytotoxicity on the U138MG glioma cell line in comparison with normal neuronal tissue.

Materials and methods

Cells and cell culture

U138MG human glioblastoma cell line was obtained from the American Type Culture Collection (Rockville, Maryland, USA). Cells were grown in culture flasks in Dulbecco's modified Eagle's medium (DMEM)/15% fetal bovine serum (FBS) (v/v) (Cultilab, Campinas, SP, Brazil) and seeded in 24-well plates (TTP plates) at densities of 1×10^4 cells/well in 500 µl medium per well. Culture cells were maintained in 5% CO_2/95% air at 37°C and allowed to grow to confluence.

Organotypic hippocampal cultures

Organotypic hippocampal cultures were prepared according to the method of Stoppini *et al.* [14]. The local Animal Care Committee approved all animal procedures used. Hippocampal slices were obtained from 6–8-day-old Wistar male rats by removing the brain, dissecting hippocampi and making transverse slices (400 µm), using a Mcllwain tissue chopper (Mickle Laboratory Engineering, Gomshall, Surrey, UK). Slices were separated in iced Hank's balanced salt solution (Gibco/BRL, Grand Island, New York, USA) supplemented with 25 mmol/l *N*-2-hydroxyl piperazine-*N*'-2-ehane sulfonic acid (HEPES), 1% fungizone and 36 µl/100 ml gentamicin, pH 7.2. Six slices were placed on one Millicell culture insert (Millicell-CM; Millipore, Bedford, Massachusetts, USA; 0.4 µm) and the inserts were transferred to a six-well culture plate (Cell Culture Cluster, Costar Cambridge, Massachusetts, USA) with 1 ml of culture medium consisting of 50% minimum essential medium (Gibco/BRL), 25% heat inactivated horse serum (Gibco/BRL) and 25% Hank's balanced salt solution, supplemented with 36 mmol/l glucose, 2 mmol/l glutamine, 25 mmol/l HEPES, 4 mmol/l $NaHCO_3$, 1% fungizone and 36 µl/ 100 ml gentamicin (final concentrations). Culture cells were maintained in 5% CO_2/95% air at 37°C. The medium was changed every 3 days and experiments were carried out after 14 days *in vitro*.

Oxygen and glucose deprivation

The induction of oxygen and glucose deprivation (OGD) was based on the method described by Strasser and Fischer [15] with some modifications [16]. Cultures were carefully rinsed twice with OGD medium composed of 1.26 mmol/l $CaCl_2$, 5.36 mmol/l KCl, 136.9 mmol/l NaCl, 0.34 mmol/l H_2PO_4, 0.49 mmol/l $MgCl_2$, 0.44 mmol/l $MgSO_4$ and 25 mmol/l HEPES, pH 7.2. Slices were left in 1 ml of this medium for 15 min and then the medium was replaced by one with the same composition, which was previously bubbled with nitrogen for 30 min. The cultures were transferred to an anaerobic chamber at 37°C in which oxygen was replaced by nitrogen, and kept in these conditions for 60 min. Slices returned to usual culture conditions for 24 h corresponding to reperfusion period. Hippocampal organotypic cultures not exposed to OGD were considered as control.

Quantification of cellular death in organotypic hippocampal cultures

Cellular damage was assessed by fluorescent image analysis of propidium iodide (PI) uptake. After a recovery period of 22 h, 7.5 µmol/l PI was added to cultures and incubated for 2 h. PI is excluded from healthy cells, but following loss of membrane integrity enters cells, binds to DNA and becomes highly fluorescent [17]. Cultures were observed with an inverted microscope (Nikon Eclipse TE 300; Nikon, Melville, New York, USA) using a standard rhodamine filter set. Images were taken and then analyzed using Scion Image software (www.scioncorp.com). The area where PI fluorescence was detectable above background levels was determined using the 'density slice' option of the software and compared with the total CA1 area to obtain the percentage of damage [18].

Quercetin treatment

Glioma cells were seeded at 1×10^4 cells/well in DMEM/15% FBS in 24-well plates. After reaching subconfluence, the cultures were exposed to quercetin (Sigma, St Louis, Missouri, USA) at concentrations of 3, 10, 30 or 100 µmol/l dissolved in DMSO (Sigma), under different timings (24, 48 and 72 h), with medium changes and quercetin replacement at each 24 h. In experiments made in hippocampal organotypic cultures, the cells received quercetin (100 µmol/l) during the OGD (60 min) and the recovery period (24 h). Control cultures were performed with DMSO (0.5% final concentration) in the absence of quercetin.

Cell counting

Glioma cells were seeded at 1×10^4 cells/well in DMEM/15% FBS in 24-well plates. At the end of the quercetin treatment, the medium was removed, cells were washed with phosphate-buffered saline (PBS) and 200 µl of 0.25% trypsin/ethylene diaminetetraacetic acid solution (Gibco/BRL) was added to detach the cells, which were counted in a hemocytometer.

Assessment of glioma cell viability

Glioma cells were seeded at 2×10^3 cells/well in DMEM/15% FBS in 96-well plates (TPP plates). Glioma cells were then exposed to different concentrations of quercetin for 48 h. Control cultures were performed with the addition of 15% FBS (cell viability control) or DMSO (vehicle control) in the absence of quercetin. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tertrazolium bromide (MTT) assay (CellTiter

96 Assay; Promega, Madison, Wisconsin, USA), in accordance with the manufacturer's instructions. Results were expressed as the percentage of cell viability against the control.

Propidium iodide assay

Glioma cells were treated with quercetin (30 or 100 μmol/l) or DMSO (control) under different timings (24, 48 and 72 h). At the end of the quercetin treatment, glioma cells were incubated with PI (7.5 μmol/l) for 1 h. PI fluorescence was excited at 515–560 nm using an inverted microscope (Nikon Eclipse TE 300) fitted with a standard rhodamine filter. Images were captured using a digital camera connected to the microscope. The results were expressed as a ratio of PI-labeled cells/total number of cells.

Caspase assay

To measure the caspase activity, glioma cell cultures (U138MG) were washed in PBS (mg/l): 0.4 Na_2HPO_4, 8 NaCl and 0.2 KCl, pH 7.4, and then lysed with 0.2% Triton X-100 in PBS for 10 min on ice. The extract was centrifuged at 10 000 *g* for 5 min and supernatant was collected. Protein concentrations of supernatant were determined by the modified Lowry procedure as described previously [19]. For each reaction, sample containing 35 μg of protein were incubated with 20 μmol/l synthetic substrate for caspase-3/7 Ac-Asp-Glu-Val-Asp-MCA (Peptide Institute, Osaka, Japan) in 100 mmol/l HEPES–NaOH pH 7.5, 10% sucrose, 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate 0.1 mg/ml BSA, 10 mmol/l DL-dithiothreitol (100 μl final volume). Caspase-3/7 cleaves the substrate between D and MCA, whose fluorescence was monitored during 90 min at 370 nm excitation/460 nm emission in a microplate fluorescence reader (Molecular Devices, Sunnyvale, California, USA) equipped with a software module for kinetic analysis (SOFTMax PRO; Molecular Devices). The fluorescence intensity was calibrated with standard concentrations of MCA, and caspase-3/7 activity was calculated from the slope of the recorder trace and expressed in picomols per milligram of protein.

Cell cycle analysis

To analyze the cell cycle, we used the method described by Overton and McCoy [20] with slight modifications. Cells were plated in six-well plates, and after 2 days they were treated with quercetin (100 μmol/l) for 24, 48 and 72 h, and subsequent to the supernatant removal, cells were washed with PBS and directly treated with a solution containing 50 μg PI, 10 mmol/l Trizma base (Sigma), 10 mmol/l NaCl, 0.7 U RNAse (Invitrogen, Carlsbad, California, USA) and 0.01% Nonidet P-40 (Sigma). After 10–30 min, data were collected of 30 000 FL2w (red-orange) pulses using the width (FL2w) and area (FL2a) parameters to exclude the doublets. Results were analyzed by Winmdi and Cylchred software (University of Wales College of Medicine, Cardiff, UK). Cells treated with DMSO were used as negative control.

Mitotic index

To measure the mitotic index, after quercetin exposure (100 μmol/l for 24, 48 and 72 h), cell cultures were washed in PBS, impermeabilized with ethanol (70%) for 10 min and incubated with PI (6.4 μmol/l) for 20 min. The presence of condensed DNA was scored as a mitotic cell and the results were expressed as a ratio of mitotic cells/total number cells.

Statistical analysis

The results are presented as mean ± SD. Data were analyzed by one-way analysis of variance, followed by Tukey's test. *P* values below 0.05 were considered for statistical significance.

Results

Quercetin decreased U138MG glioma cell growth and viability

In order to investigate the effect of quercetin on proliferation/viability of U138MG glioma, the cell cultures were treated with quercetin, counted and an MTT assay was performed. Exposure of glioma cells to quercetin for 24, 48 and 72 h resulted in a cell number decrease in a time-dependent fashion at concentrations of 30 μmol/l (22, 58, 74%) and 100 μmol/l (31, 70, 76%), respectively (Fig. 1). Parallel to this result, at the same dose interval (10, 30, 50 or 100 μmol/l), quercetin treatment also resulted in a reduction of cell viability (30, 42, 52 and 52%, respectively), as evidenced by a decreased ability of glioma cells to reduce MTT when measured in the 48 h following exposure to quercetin (Fig. 2). The reduction in MTT staining suggests not only glioma cell damage, but also a decrease in cell proliferation when compared with the control.

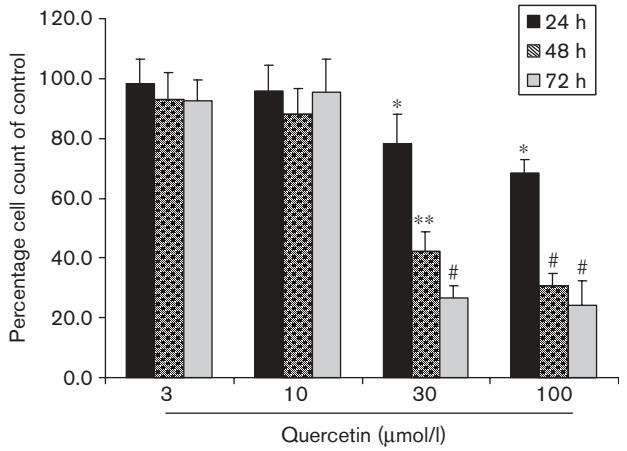
Cell death induced by quercetin

To determine whether the suppression of glioma cell proliferation was due to the induction of necrosis, glioma cells were treated with quercetin (30 or 100 μmol/l) in different timings (24, 48 and 72 h) and then analyzed for cell membrane permeability by PI. As shown in Fig. 3, PI incorporation was higher in cells treated with quercetin, suggesting that quercetin exposure resulted in a loss of membrane integrity, which is an indication of cell death.

Quercetin treatment induced activation of caspase-3/7

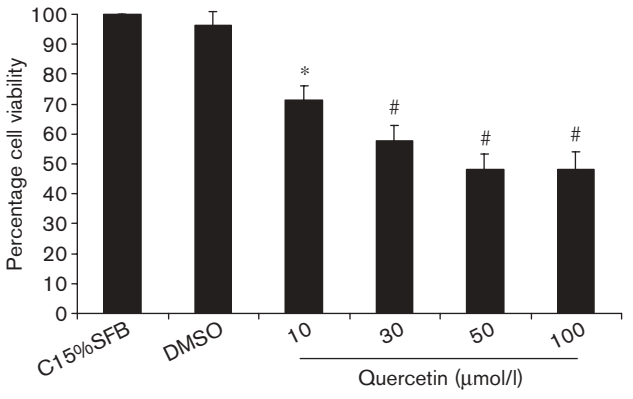
Flavonoids, especially quercetin, may modulate the apoptosis pathway, interacting directly with mitochondria, causing the release of cytochrome *c* and the subsequent activation of caspase-3/7 [12]. To investigate whether the treatment of U138MG glioma with quercetin resulted in caspase-3/7 activation, cell extracts of glioma cell cultures treated with quercetin (30 or 100 μmol/l) for 24, 48 and 72 h were tested for caspase

Fig. 1



Effect of quercetin on growth of U138MG glioma cells. Cells were treated for 24, 48 and 72 h with quercetin (3, 10, 30 or 100 μmol/l) or dimethylsulfoxide (DMSO) (control), and cells were counted in a hemocytometer as described in Material and methods. Cell count in samples treated with DMSO was considered 100% of cell number. Data are the mean ± SD. $P < 0.001$ as determined by one-way analysis of variance followed by Tukey's test. The symbols *, ** and # indicate statistical difference from control and from all other treatments. Absolute cell numbers for the control values (control 24 h: 32 542 ± 3700; control 48 h: 64 830 ± 3000; control 72 h: 90 700 ± 4000).

Fig. 2



Effects of quercetin on U138MG glioma cell viability assessed by the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tertrazolium bromide (MTT) assay. Cell cultures were exposed to quercetin (10, 30, 50 or 100 μmol/l) for 48 h, and the cell viability was assessed by the MTT assay as described in Material and methods. Glioma cultures treated with 15% FBS was taken as 100% of cell viability. Data are the mean ± SD. $P < 0.05$ as determined by one-way analysis of variance followed by Tukey's test. The symbols * and # indicate statistical difference from control and from the other treatments (Control absorption value = 1.540 ± 0.071). DMSO, dimethylsulfoxide.

activity using a specific synthetic substrate (Fig. 4). Caspase activation was detected at a dose of 100 μmol/l (Fig. 4), and this proapoptotic effect initiated at 48 h and remained up to 72 h after exposure. Quercetin at a concentration of 30 μmol/l was not able to induce caspase activation at any time of treatment (data not shown).

Quercetin promoted G_2 phase arrest in glioma cells

To explore the influence of quercetin on cell cycle progression, the proportion of cells in the G_1, S and G_2/M phases was determined in the presence or absence of quercetin (100 μmol/l) after 24, 48 and 72 h of treatment. The flow cytometry analyses using PI for DNA labeling showed that quercetin induced a time-dependent blockage on the relative content of cells in the G_2/M phase (Fig. 5). The accumulation of cells in G_2/M may suggest a block at either the G_2 entry or the anaphase checkpoint and, in order to investigate whether the accumulating tetraploid cells have condensed DNA, we performed a staining with PI, and the mitotic index of these cultures was evaluated. As shown in Fig. 6, quercetin treatment resulted in a decrease of mitotic index of around 57% compared with control cells. This effect started after 24 h of treatment and remained constant up to 72 h, indicating that the block is at the entry of G_2 and not at the anaphase checkpoint.

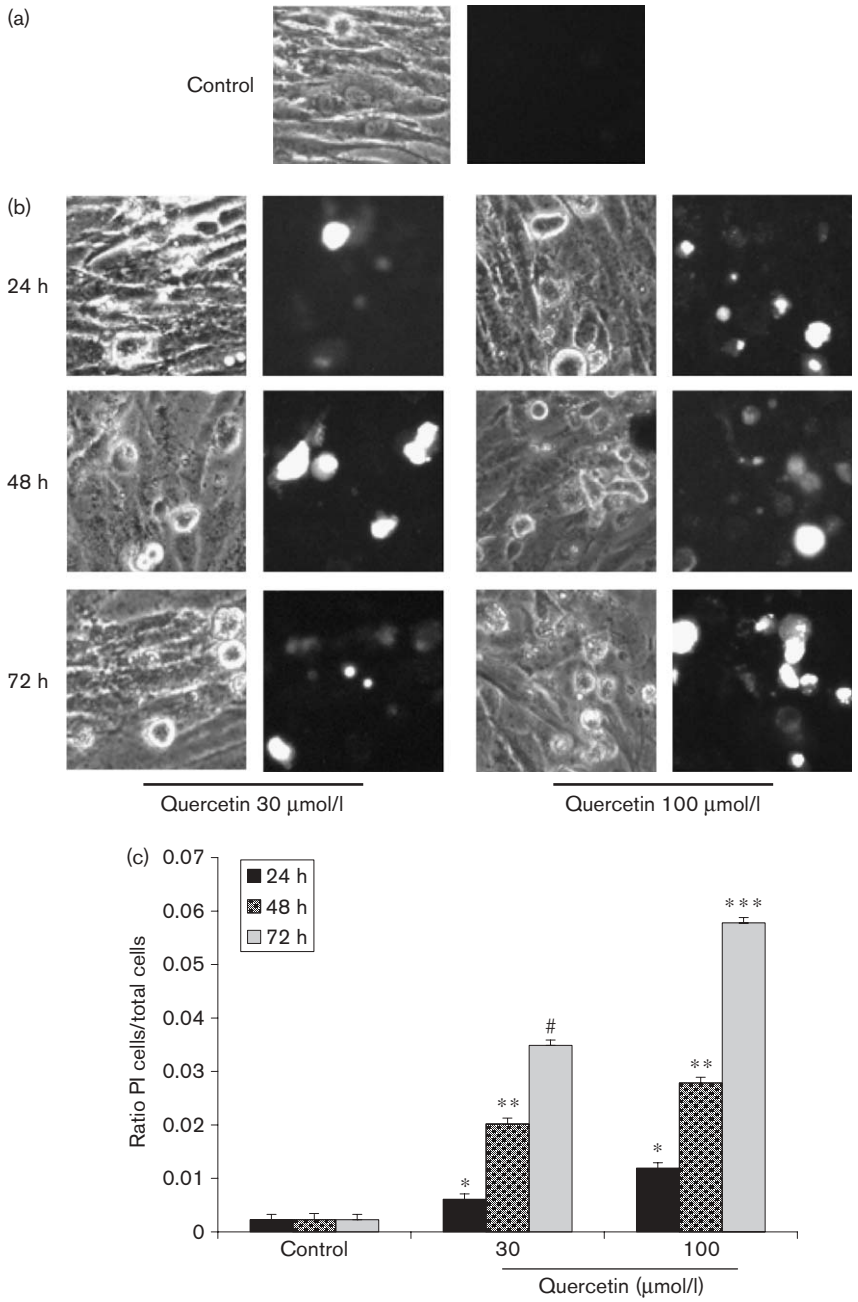
Quercetin protected organotypic hippocampal culture from oxygen and glucose deprivation-induced neurotoxicity

Flavonoids have been shown to inhibit cell growth and induce apoptosis in various human cancer cells with little or no effect on normal cells [21]. Moreover, studies have suggested that diet-derived flavonoids may play a beneficial role in the prevention of neurodegeneration [22]. Thus, to evaluate the selective cytotoxic effect of quercetin on glioma cell cultures, hippocampal organotypic cultures were treated with quercetin (100 μmol/l) for 24 h and the cell death was observed by PI uptake (Fig. 7). In addition, hippocampal organotypic cultures were exposed to OGD and the potential of quercetin to protect against ischemic insult was estimated. Firstly, it is important to note that quercetin *per se* did not promote hippocampal organotypic culture damage (control quercetin 100 μmol/l). At approximately 24 h after OGD had finished, around 80% of the control organotypic hippocampal cultures were damaged (DMSO-treated cultures). Whereas organotypic hippocampal cultures were treated with quercetin (100 μmol/l) during and after the OGD period, neuronal injury was reduced to 35% (Fig. 7a and b), indicating that quercetin-treated cultures were less susceptible to OGD-induced damage.

Discussion

Cancer is a growing health problem around the world. Recent studies have estimated that more than two-thirds of human cancers could be prevented through appropriate lifestyle modification. Epidemiological and statistical data have pointed out that about 35% of human cancer mortality is attributable to diet [23]. It is well accepted that a diet rich in fruits and vegetables is associated with

Fig. 3



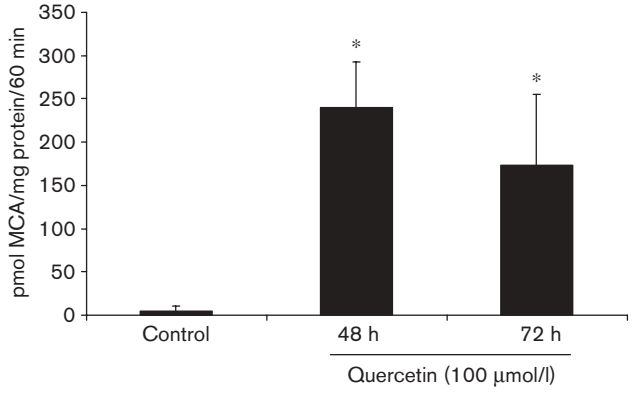
Representative pictures and analysis of U138MG cell culture stained with propidium iodide (PI). U138MG glioma cells were treated with (a) dimethylsulfoxide (DMSO) (control) and (b) 30 or 100 μmol/l of quercetin for 24, 48 and 72 h. (c) Quantitative analysis of damage in DMSO (control) or quercetin treatments. Data are the mean ± SD. $P < 0.05$ as determined by one-way analysis of variance followed by Tukey's test. The symbols *, **, # and *** indicate statistical difference from the control and from all other treatments. Cellular death was analyzed by PI incorporation that was visualized using a Nikon inverted microscope and the results were expressed as ratio PI-labeled cells/total cell well.

a reduced risk of cancer [24,25], although a recent study indicated that flavonoids present protective effects only against lung cancer [26]. Among the dietary phytochemicals that have been found to exert chemopreventive effects in cancer, the dietary flavonoid quercetin is thought to be a good nominee.

In the present study, the ability of quercetin to inhibit the proliferation of U138MG glioma cell culture was observed. Results from cell counting showed that quercetin inhibited the proliferation of glioma cells. In addition, the glioma cell damage was estimated by the MTT assay and the data showed that quercetin reduced

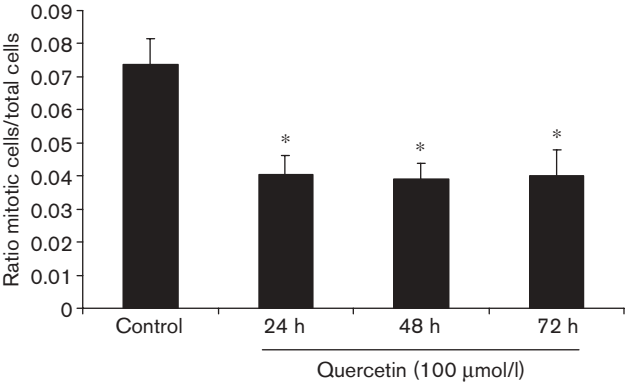
the cell viability of glioma. These results are in accordance with data suggesting that quercetin have a growth-inhibitory effect on distinct tumor cell lines [27]. Quercetin promotes release of mitochondria-accumulated Ca^{2+}, which is critical for cell function, and may favor both apoptosis and necrosis death [28]. Thus, in order to investigate whether the antiproliferative effect of

Fig. 4



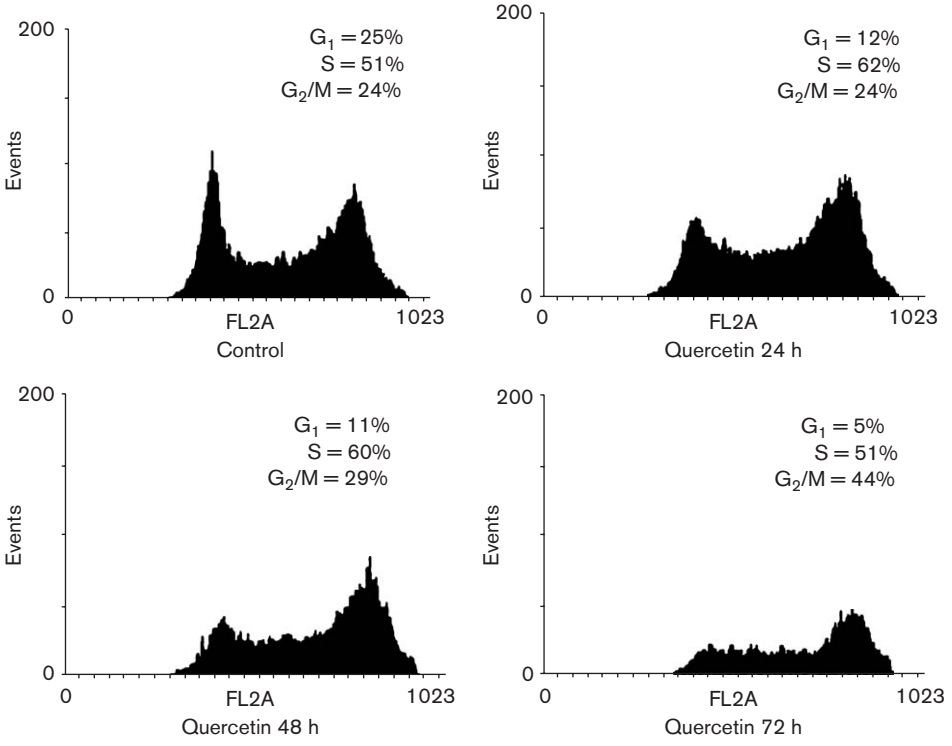
Caspase-3/7 activity in U138MG glioma cell. Cells were treated with dimethylsulfoxide (DMSO) (control) or quercetin (100 imol/l) for the indicated times and caspase-3/7 activities were determined by fluorescence intensity examination. DMSO (control), 48 and 72 h represent quercetin treatment for 48 and 72 h, respectively. Data represent the means of three independent experiments performed in duplicate ± SD. *Indicates difference from control $P < 0.05$ as determined by one-way analysis of variance followed by Tukey's test.

Fig. 6



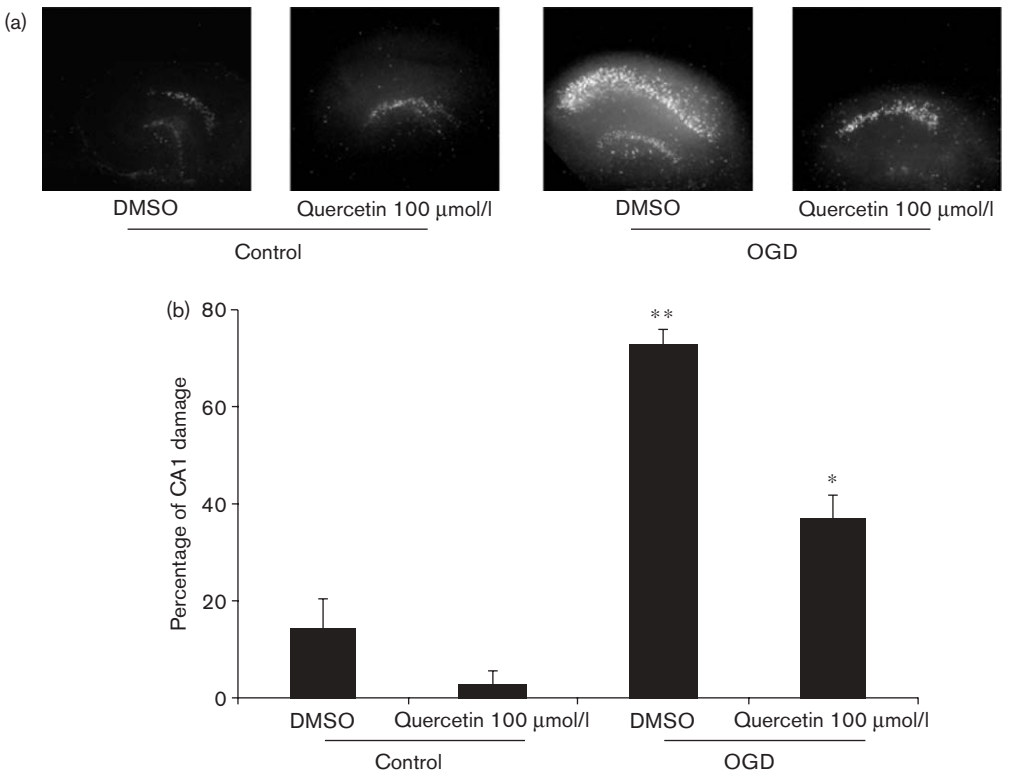
Effect of quercetin on the mitotic index on U138MG glioma cells. Cells were treated as in Fig. 5 and mitotic index assay was performed at the indicated times. Data represent the means of three independent experiments performed in quintuplicate ± SD. *Indicates difference from control $P < 0.05$ as determined by one-way analysis of variance followed by Tukey's test.

Fig. 5



Effect of quercetin on cell cycle distribution of U138MG glioma cells. Cells were treated with dimethylsulfoxide (control) or quercetin (100 μmol/l) and subjected to flow cytometry cell cycle analyses at the indicated times as described in Material and methods. Values are the relative number of cells in the G_1, S and G_2/M phases of cell cycle. Data are from three experiments.

Fig. 7



Effect of quercetin on organotypic hippocampal slice cultures. (a) Representative pictures of organotypic hippocampal cultures at 14 days. Cell cultures were treated with dimethylsulfoxide (DMSO) (vehicle control) or quercetin at day 14 for 24 h. Control: neuronal cultures not exposed to oxygen and glucose deprivation (OGD); OGD: neuronal cultures exposed to oxygen glucose deprivation. Cellular death was analyzed by propidium iodide incorporation, which was visualized using a Nikon inverted microscope (at $\times 40$ magnification). (b) Quantitative analysis of damage in control and OGD organotypic slices treated with vehicle (DMSO) or quercetin. Data represent the means ± SD of four independent experiments performed in duplicate. Data were analyzed by one-way analysis of variance followed by Tukey's test. **Significantly different from vehicle-treated DMSO and quercetin-treated of control cultures ($P<0.001$). *Significantly different from all other groups ($P<0.01$).

quercetin was related to necrotic or apoptotic cell death, the PI uptake by glioma cells and the caspase activation assay were performed following quercetin exposure. Our results showed that quercetin treatment induced cell necrosis at the onset of treatment (24 h), while inducing caspase activation only at a higher dose and after 48 h of treatment. To sum up, these results suggest that the cell death found at treatment onset and at lower concentrations of quercetin could be a consequence of pathways other than caspase-dependent apoptosis. Recently, it was demonstrated that quercetin was very effective in interacting with mitochondrial membrane, decreasing both its fluidity and ATP production, suggesting a potential for necrosis induction [28]. This quercetin/mitochondria interaction can partially explain the cell necrosis found in glioma cells. Moreover, according to previous studies, which showed apoptosis induction by flavonoids, activation of caspase-3/7 was observed in glioma cells exposed to a high dose of quercetin (100 µmol/l) (Fig. 4). The proapoptotic effect found in the present study is likely to be a consequence of inhibition of PI3K, following the reduction of Akt phosphorylation, which leads to release of cytochrome *c*, formation of apoptosome and subsequent caspase-3/7 activation [12]. The inhibition of PI3K–Akt pathway by quercetin may be a useful tool of glioma cell treatment; as U138MG does not express PTEN, the phosphoinositide-3-phosphate phosphatase results in a constitutively active Akt. It is one of the major events in gliomagenesis, which is correlated with poor prognosis for patients [29].

Cell cycle pathway abnormalities play a central role in gliomagenesis. As flavonoids were described to inhibit the cell cycle progression of tumor cells [30], and quercetin may modulate the expression of cyclins and cyclin-dependent kinases [10], we investigated the possible effect of quercetin on cell cycle of U138MG glioma cultures. Quercetin induced arrest in the G_2 phase of the cell cycle in a time-dependent fashion and, consequently, a decrease in the mitotic index. These results indicate that quercetin blocks glioma cell progression at the G_2

checkpoint and not at the anaphase alignment checkpoint. The G_2/M checkpoint represents a second distinct time in the cell cycle that is controlled by critical cell cycle events. A major regulator of the G_2/M checkpoint is the protein product of the tumor suppressor p53. The p53-mediated checkpoint response makes the choice between cell cycle arrest and cell death. Mutations on the p53 gene are common in a variety of mammalian tumors, including the U138MG glioma cell line [31]. A study performed in human breast carcinoma (MDA-MB468 cells) demonstrated that quercetin can modulate the cellular neoplastic phenotype, downregulating the expression of mutant p53 protein [32]. Thus, we assume that both arrests in G_2 and apoptosis activation mediated by quercetin in U138MG glioma cells might be due to a quercetin/p53 interaction. Further studies, however, are necessary to give support to this hypothesis.

In addition, the cytotoxic effect of quercetin on neuronal culture cells was evaluated. An important outcome of the present study is that the cell death induced by quercetin found in glioma was not observed in the organotypic cultures. Moreover, under our assay conditions, quercetin was effective to protect the hippocampal organotypic culture from ischemic insult while, under the same experimental conditions, this flavonoid induced proliferation/viability decrease, necrosis/apoptosis activation and arrest in cell cycle in U138MG glioma cell cultures. These results suggest that quercetin not only has a cytotoxic effect on tumoral cells, but may also protect the normal cells against ischemia. It is important to mention that excitotoxic neuronal cell death may play a very important role in the glioma etiology [33]. Quercetin has affected tumor survival negatively and neuronal survival positively; thus, this drug may be considered as a good candidate for therapeutic intervention in gliomas.

Although quercetin is one the most frequently studied flavonoids, the range of concentrations that delimits the beneficial and deleterious effects remains unclear. Some studies suggest that quercetin presents a biphasic effect: low concentrations (10–25 μmol/l) may protect against neurodegenerative disease, whereas high concentrations (50–250 μmol/l) induce cell cytotoxicity [34,35]. On the other hand, studies with 'high' concentrations of quercetin (25–100 μmol/l) pointed to neuroprotective and antiapoptotic effects on normal cell cultures [36–38]. One possible explanation for these controversial effects elicited by quercetin is that it presents both prooxidant and antioxidant characteristics, and the resultant effect is closely related to the redox state of the cell [39], signaling transduction pathways or cell type. The quercetin neuroprotector effect demonstrated here could involve both antioxidant properties and modulatory actions in prosurvival signaling pathways, such as c-Jun N-terminal kinase-mediated and extracellular signal-regulated kinase-mediated actions [37]. Additional experiments, however, should be performed to elucidate the cell pathways by which quercetin induces antiproliferative effects in glioma cells, while protecting the hippocampal organotypic cultures from damage.

In summary, the present study demonstrates that quercetin has an antiproliferative effect in U138MG glioma. Among the antitumoral effects elicited by quercetin, we demonstrated a decrease of glioma cell proliferation and viability; necrosis death and, at high quercetin concentrations, apoptosis induction; arresting in the G_2 checkpoint, followed by a decrease of mitotic index. Therefore, the ability of quercetin to retard the growth of U138MG glioma cells should be recognized as a combination of distinct signaling pathways, which is highly desirable for optimizing cancer therapy. Furthermore, we demonstrated that quercetin is able to protect the hippocampal organotypic cultures from ischemic damage. Although quercetin is one of the most studied flavonoids, its availability to tissues is still unknown. After oral ingest, quercetin is quickly metabolized by enzymes in the small intestine and later metabolized by the liver, resulting in low micromolar plasmatic concentrations in humans [40]. As the antiproliferative effect of quercetin was obtained at high micromolar concentrations, we suggest that this pharmaceutical formula could overcome this problem in an attempt to increase quercetin bioavailability. Taken together, ours results imply that quercetin may be considered a potential candidate for both cancer prevention and treatment, without the undesirable side-effects of conventional chemotherapy. Further investigations using *in vivo* glioma model should be helpful to confirm the distinct effects of quercetin in normal versus tumoral cells, as well as to determine the appropriate levels of quercetin for optimum cancer chemopreventive actions.

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