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Quercetin and anti-CD95(Fas/Apo1) enhance apoptosis in HPB-ALL cell line

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Abstract Several malignant cell lines are resistant to CD95-(Apo1/Fas)-mediated apoptosis, even when the CD95 receptor is highly expressed. Sensitivity to CD95-induced apoptosis can be restored using different molecules. In this study, we showed that quercetin, a naturally occurring flavonoid, in association with the agonistic anti-CD95 monoclonal antibody, increases DNA fragmentation and caspase-3 activity in HPB-ALL cells. These cells have been selected for their known resistance to CD95-induced apoptosis. At molecular level, quercetin lowers the level of intracellular reactive oxygen species, reduces mitochondrial transmembrane potential, thereby leaving the expression of CD95 receptor unchanged.

Key words: Quercetin; CD95/Apo1/Fas; Apoptosis; Flavonoid; HPB-ALL cell line

1. Introduction

A great deal of evidence indicates that cancer is the result of a reciprocal interaction between genetic susceptibility and environmental factors. In this respect, dietary habit plays a fundamental role. Following the recent publication from the American Institute for Cancer Research [1], a 'correct' diet could decrease the cancer rate by as much as 20%. For certain cancers, the correlation is even more dramatic. Recent studies have focused on the activity of non-nutritional compounds, present in the diet, that can prevent the occurrence of degenerative diseases such as cancer. This class of molecules, generally known as chemopreventers [2] includes some vitamins and food polyphenols. Flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages such as tea and wine [3]. A large number of epidemiological and molecular studies suggest that a daily intake of flavonoids could reduce the incidence of several types of cancers [4,5].

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is the major dietary flavonoid particularly abundant in fruit and vegetables [3], with a daily intake of 16 mg in European countries [6]. Some food, such as wine and tea, may contain a concentration of quercetin up to 150 μM [7]. This molecule has been reported to inhibit the growth of various cell lines derived from human cancers [8,9]; it blocks the cell division cycle at different points depending on the cell type and tumor origin: at G2/M transition [8–10], or G1/S [11,12]. In addition, quercetin exerts a well documented pro-apoptotic activity [9,13–15] that generally follows cell cycle block. This suggests that the failure of malignant cells to pass quercetin-dependent cell cycle arrest can trigger apoptosis. However, despite the large number of data on the antiproliferative and pro-apoptotic effects of quercetin, its mechanism of action is still unknown. Quercetin seems to modulate cellular neoplastic phenotype by down-regulating the expression of mutated H-*ras* and *p53* [8], c-*myc* and K-*ras* [16], or up-regulating $p21^{WAF1/CIP1}$ [14]. Alternatively, quercetin activity might be related to its ability to inhibit various protein kinase and serine/threonine protein kinases [17–19]. Despite these data, several controversial results have been published on the real beneficial effect of quercetin [2,20,21]. Concerns have been expressed on the presence of quercetin and other flavonoids in food as potential genotoxic hazard since they can induce p53-mediated apoptosis in non-tumor cells [10]. In normal cells, quercetin inhibits apoptosis via suppression of the tyrosine kinase/c-Jun/AP-1 pathway triggered by oxidative stress [22].

CD95 and its ligand, CD95-L, play a key role in several types of physiological apoptosis [23,24]. CD95-mediated apoptosis can be triggered by the natural ligand of the receptor, CD95-L expressed on several cell types included killer cells, or by agonistic antibodies such as anti-CD95 [23,25]. The best understood signal transduction pathway of CD95-mediated apoptosis involves activation of FADD and caspase-8 that triggers the protease cascade leading to the activation of downstream effector caspases such as caspase-3 -6 and -7 depending on cell type origin [26,27]. Activation of CD95 pathway also induces a mitochondria-dependent apoptotic event [28–30]. However, different cell types utilize either of the two CD95 signaling pathways depending on the specific type [26,27].

In order to study the effect of quercetin in CD95-induced apoptosis, we chose HPB-ALL cell line derived from a human tymoma [31]. These cells are characterized by unusual resistance to apoptosis induced in vitro, and are derived from a tumor type with alteration in the CD95 pathway of signal transduction [25]. The majority of ALL (acute lymphoblastic leukemia) are resistant to CD95-triggered apoptosis, resembling the relative resistance of most human thymocytes,

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Abbreviations: ROS, reactive oxygen species; $\Delta\Psi_m$, mitochondrial transmembrane potential; Z-DEVD-AFC, carbobenzoxy-Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin; $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; DCFDA, 2′,7′-dichlorofluorescin diacetate; DMSO, dimethylsulfoxide; TUNEL, TdT-mediated dUTP nick end labelling

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although they express significant (or high) levels of CD95. Explanations for this apparent contradiction might include defects in the receptor, or ligand, or other molecules involved in the apoptotic signaling [25].

In this report, we showed that quercetin is able to sensitize malignant cells normally resistant to CD95-mediated apoptosis. Our results suggest that the molecule sustains this role by acting at several levels on the signal transduction pathway triggering CD95-mediated apoptosis.

2. Materials and methods

2.1. Cell culture

Human tymoma-derived HPB-ALL cells [31] were cultured in RPMI medium (Gibco-BRL) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 1% L-glutamine in a 5% CO_2 incubator at 37°C. Cells were commonly grown in 100 mm Petri dishes and the medium was changed every 48 h.

2.2. Detection of viability and cell death

To assay the effect of quercetin on cell growth, viability was determined by trypan blue exclusion. Percentage of viable cells was estimated after counting 100 cells for each sample. The cytotoxicity of anti-CD95, quercetin or both on HPB-ALL was assayed by neutral red viability test uptake. Cells were seeded ($2–3\times10^5$/well) in a 12 multiwell plate and incubated with medium containing 0.1% DMSO (control), 50 ng/ml anti-CD95, 50 μM quercetin dissolved in 0.1% DMSO for 12 h. Subsequently, neutral red (0.066% v/v in the culture medium) was added, and cells incubated for 3 h. To estimate the incorporation of dye, cells were incubated for 15 min with a lysis buffer (50 mM Tris-HCl pH 7.4; 150 mM NaCl; 5 mM dithiothreitol, and 1% Triton X-100) containing 1% acetic acid and 50% ethanol. Finally, absorbance was measured spectrophotometrically at 540 nm. Apoptosis in HPB-ALL cells was determined using an in situ cell death detection kit (Boehringer Mannheim). Fixed cells with fluorescein-labelled DNA strand breaks were subsequently stained with propidium iodide and observed at confocal microscopy (Leica TCS SP). Percentage of apoptotic cells was estimated after counting at least 500 cells for each sample.

2.3. Caspase-3 activity

To study the early events of apoptosis in HPB-ALL, we assayed caspase-3 (CPP32/caspase-3/Apopaine) protease activity using a commercially available Apopain assay kit (BioRad). Briefly, cells were incubated with medium containing 0.1% DMSO, 50 ng/ml anti-CD95, 50 μM of quercetin, or both for 6 h, and samples were assayed at 37°C for 30 min in the presence of the specific conjugated substrate Z-DEVD-AFC (carbobenzoxy-Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin), according to the manufacturer's instructions. Upon proteolytic cleavage of the substrate by caspase-3, the free fluorochrome AFC was detected by a spectrofluorimeter (Perkin Elmer LS 50B) with excitation and emission setting at 395 nm and 540 nm, respectively. To quantify enzymatic activity, we made a standard curve of AFC. Caspase-3 specific activity in each sample was calculated as μmoles of AFC produced/min/μg protein at 37°C at saturating substrate concentrations (49 μM).

2.4. Immunoblotting

For Western blotting analysis, cells ($4–5\times10^6$ for each sample) were lysed in 100 μl lysis buffer (50 mM Tris pH 7.6; 150 mM NaCl; 5 mM EDTA; 1% NP-40; 0.5 mM dithiothreitol; 10% glycerol containing 100 μg/ml phenylmethylsulfonyl, and a cocktail of protease inhibitors (complete' by Boehringer). Proteins in the cell lysates (20 μg/lane) were separated by 15% SDS-PAGE before blotting onto PVDF (polyvinylidene fluoride, Millipore) membrane. The membrane blot was incubated with anti-CD95 antibody (CH11, Immunotech, Marseille, France) diluted 1:500. Following incubation with a horseradish peroxidase-linked secondary antibody, CD95 was revealed using a chemiluminescence detection kit (ECL-Plus, Amersham). For quantitative analysis of protein expression, the same membrane was stripped and probed with anti-α-tubulin monoclonal antibody (Sigma). Densitometric analysis of the bands was performed using GEL DOC 2000 system (BioRad).

2.5. Cytofluorimetric analysis of $\Delta\Psi_m$

Variations of the mitochondrial transmembrane potential ($\Delta\Psi_m$) during cellular apoptosis were studied using the cationic lipophilic dye 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) [32] (Molecular Probe). This cyanine dye accumulates in the mitochondrial matrix under the influence of $\Delta\Psi_m$. HPB-ALL cells were incubated with different reagents for 4.5 h, and treated with 100 μl of 0.1 μM $DiOC_6$ for 30 min. $DiOC_6$ membrane potential related fluorescence was recorded using flow cytometry (Becton Dickinson).

2.6. Reactive oxygen species (ROS) measurement

To detect intracellular ROS production in HPB-ALL cells, we used a spectrofluorimetric method [33]. ROS production was assayed using 2′,7′-dichlorofluorescin diacetate (DCFDA, Molecular Probe), a non-fluorescent compound that freely permeates cells. Cells (1.5×10^6/ml) were incubated with 50 μM of quercetin at different times, and incubated with 10 μM DCFDA for 30 min.

3. Results

3.1. Effect of quercetin on cell growth

In order to evaluate the capability of quercetin to induce apoptosis in HPB-ALL cell line, we first tested the effects of the molecule on cell growth. Fig. 1 shows that at a concentration up to 200 μM, quercetin was not toxic until 12 h of treatment compared to untreated cells. However, after a prolonged exposure (24 h), HPB-ALL presented a dose-dependent decrease in cellular viability as measured by trypan blue exclusion assay, indicating a general cytotoxic effect of quercetin. Based on these data, in our experiments we used a concentration of 50 μM quercetin, and we limited our observation at time points shorter than 12 h to avoid the possible toxic effects of the molecule.

3.2. Sensitivity to CD95-mediated apoptosis in HPB-ALL

To determine sensitivity of HPB-ALL to CD95-mediated apoptosis, we measured their dose dependence response to the agonistic antibody CH11 able to induce apoptotic cell death in different systems [34,35]. In HPB-ALL, anti-CD95 decreased 20% cell viability starting from a concentration of 50 ng/ml, one which falls within the range of that active in other systems [34,35] (Fig. 2). The maximal effect of anti-CD95-induced apoptosis was observed at a concentration 10-fold higher (40% decrease in cellular viability compared to untreated and IgM treated cells). Therefore, HPB-ALL cells were sensitive to 50 ng/ml anti-CD95-induced apoptosis, while saturation of CD95 receptor was detectable starting

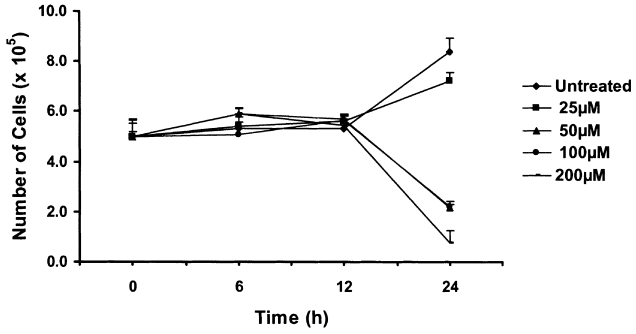

Fig. 1. Effect of quercetin on cell growth of HPB-ALL cell line. Cells were plated at a density of 5.0×10^5, and treated with the indicated concentration of quercetin. After trypan blue staining, the number of viable cells was determined at different time intervals after the onset of the treatment. Results represent the average of three experiments. Bars represent standard error.

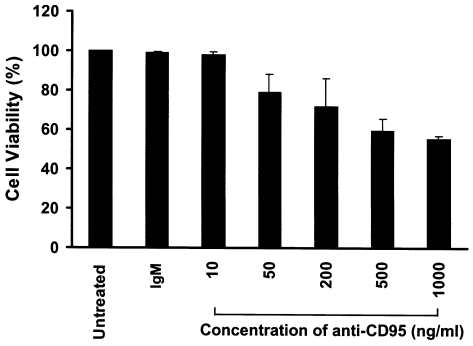

Fig. 2. Sensitivity of HPB-ALL cells to CD95-mediated apoptosis. HPB-ALL were incubated at the density reported in Fig. 1 for 12 h with the indicated concentration of anti-CD95 antibody. After incubation, cells were harvested and cell viability determined by neutral red assay. IgM (1000 ng/ml) indicates a control antiserum. The data are representatives of three independent experiments in duplicate. Bars represent standard error.

from a concentration of 500 ng/ml of antibody. In the light of the data reported in Fig. 2, we used in our experiments a concentration of anti-CD95 of 50 ng/ml. This value corresponded to a decrease in cell viability related to the pro-apoptotic activity of the antibody.

3.3. *Effect of quercetin and anti-CD95 antibody on CD95-induced apoptosis*

To investigate the effect of quercetin on CD95-induced apoptosis, we incubated HPB-ALL cells with 50 ng/ml anti-CD95 antibody in the presence of 50 μM quercetin for 12 h, and then we analyzed apoptotic cells as described in Section 2. Untreated HPB-ALL cells (Fig. 3A) and those treated solely with quercetin (not shown) displayed a number of apoptotic elements lower than 6% as determined by TUNEL (TdT-mediated dUTP nick end labelling) assay (Fig. 3C). Incubation with CH11 increased the number of apoptotic elements to about 12% (Fig. 3C). When quercetin and CH11 were added

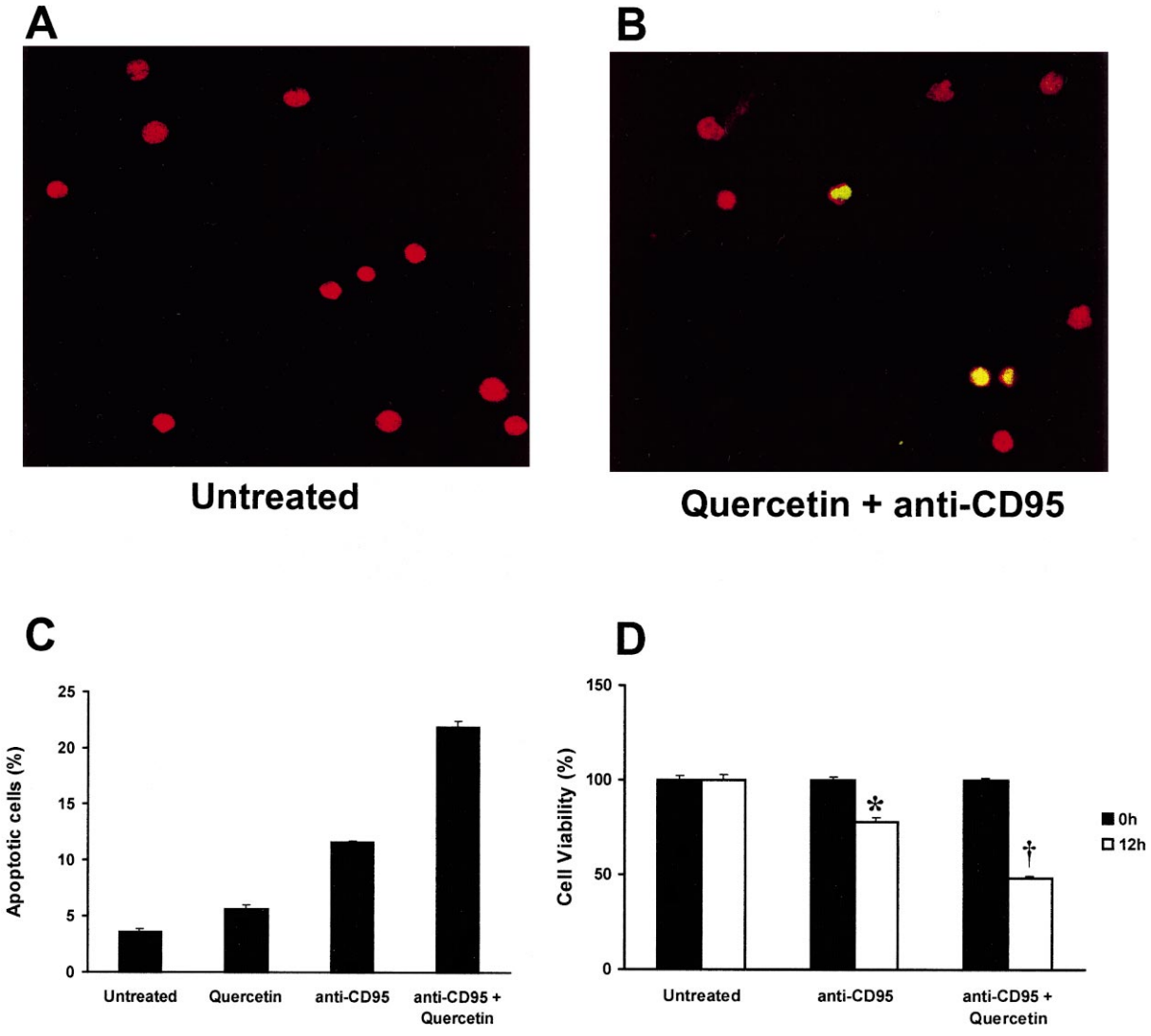

Fig. 3. Associative effect of quercetin and anti-CD95 in CD95-mediated apoptosis. HPB-ALL cells (1×10^6) were incubated for 12 h in the presence of 50 μM quercetin and 50 ng/ml anti-CD95 monoclonal antibody (CH11). DNA fragmentation was detected microscopically by TUNEL test (A, B). The average of two experiments is shown, and at least 500 cells were counted per experiment (C). In panel D, cell viability measured by trypan blue exclusion is shown. The data are representatives of two independent experiments in duplicate. Bars represent standard error. In panel C, ANOVA: $P<0.001$. In panel D, ANOVA: $P<0.01$. *$P<0.05$ vs. [untreated] and [anti-CD95+quercetin treated] cells. †$P<0.05$ vs. [untreated] and [anti-CD95 treated] cells.

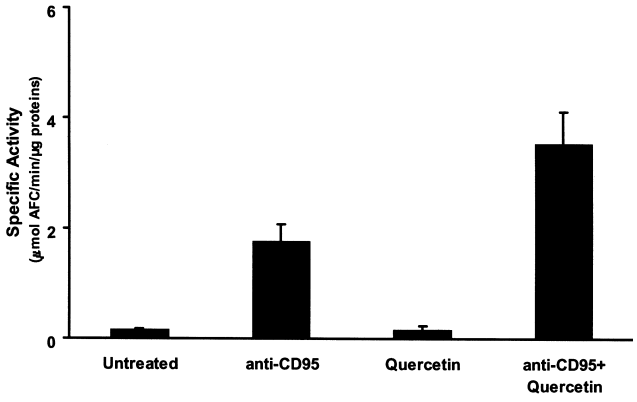

Fig. 4. Effect of quercetin on caspase-3 protease activity of anti-CD95 treated cells. HPB-ALL cells were lysated and caspase-3 enzymatic activity measures as described in the manufacturer's protocol. Each experiment was performed starting from $4–5\times10^6$ cells. The amount of cell lysate tested in each assay was approximately 5–10 μg. The data are representatives of three independent experiments. Bars represent standard error. ANOVA: $P<0.001$.

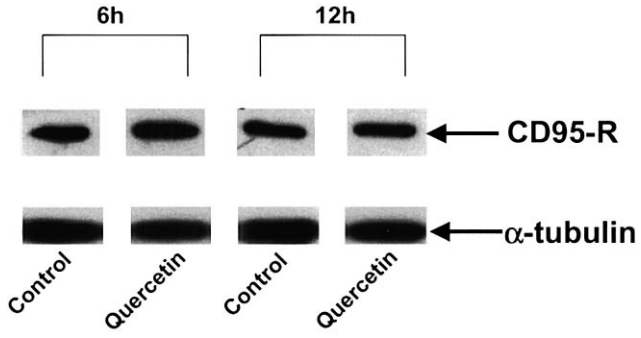

Fig. 5. CD95 expression following quercetin treatment of HPB-ALL cells. Immunoblotting analysis of CD9 receptor performed using anti-CD95 monoclonal CH11. Each lane contains 20 μg of cell extract. After the first immunoblotting the PVDF membrane was stripped and re-probed with a control antibody anti-α-tubulin.

together, apoptosis increased up to 22% (Fig. 3B and C). To confirm the associative effect of quercetin and anti-CD95 in CD95-induced apoptosis, we measured changes in cell viability using trypan blue assay. Fig. 3D shows a decrease in cell viability of 50% when quercetin and anti-CD95 were used together compared to the 20% determined by anti-CD95 antibody.

3.4. Effect of quercetin and anti-CD95 on caspase-3 activity

To characterize the apoptotic pathway triggered by quercetin in association with anti-CD95 antibody, we measured a different marker of apoptotic induction downstream to CD95, such as caspase-3 activity. HPB-ALL cells showed a clear increase in caspase-3 activity after treatment with anti-CD95 antibody (Fig. 4). However, this enzymatic activity was significantly enhanced when quercetin was coupled to CH11; in fact, caspase-3 activity increased 1.5-fold with respect to CH11 treated cells (Fig. 4).

3.5. Effect of quercetin on CD95 expression

In order to investigate the possible mechanism(s) responsible for the observed associative effect of quercetin and anti-CD95 in mediating apoptosis, we hypothesized that a change

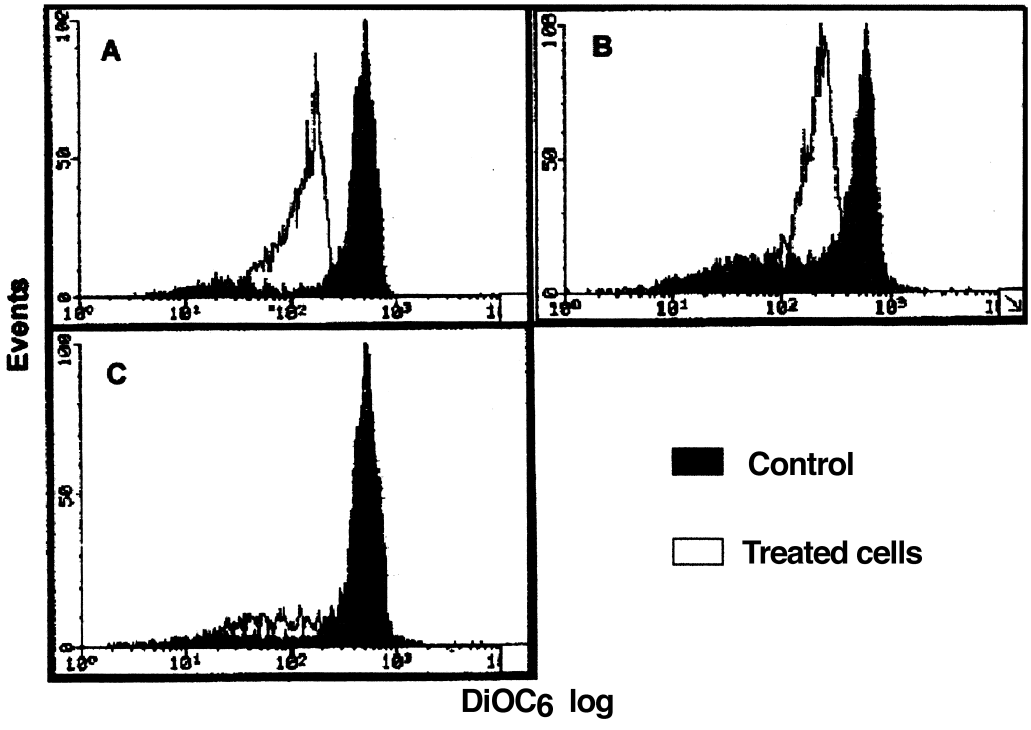

Fig. 6. Changes in $\Delta\Psi_m$ in HPB-ALL due to quercetin treatment. HPB-ALL cells (1×10^6) were incubated with 50 μM quercetin (A), 50 μM quercetin plus 50 ng/ml anti-CD95 (B), and 50 ng/ml anti-CD95 (C). All the experiments were performed for 4.5 h. Drop in $\Delta\Psi_m$ was measured by loading cells with the fluorochrome $DiOC_6$ and subsequent flow cytometric analysis.

in the expression of CD95 receptor due to quercetin treatment could explain the increased sensitivity of cells to anti-CD95. Flow cytometric analysis of CD95 expression in HPB-ALL indicated that quercetin treatment for several hours (1–4) did not induce any significant change in the expression of the receptor (data not reported). To further confirm these data, we performed an immunoblotting analysis of CD95 receptor after prolonged exposure to quercetin (6–12 h) using CH11 antibody. Fig. 5 clearly shows no change in CD95 expression.

3.6. Effect of quercetin on mitochondrial transmembrane potential

The loss of mitochondrial transmembrane potential ($\Delta\Psi_m$) associated with CD95-mediated apoptosis, has been described in different cell lines [27,36]. Therefore, we hypothesize that quercetin could enhance CD95-mediated apoptosis by acting at the level of $\Delta\Psi_m$. To verify whether this mechanism was active, we loaded HPB-ALL cells treated with quercetin with the fluorochrome $DiOC_6$ following measurement of $\Delta\Psi_m$ by flow cytometric analysis (Fig. 6). Cells incubated with quercetin for 4.5 h showed a striking reduction in $\Delta\Psi_m$ (Fig. 6A), more evident than that observed in cells incubated with anti-CD95 (Fig. 6C), and comparable to that detectable in cells incubated with quercetin and anti-CD95 together (Fig. 6B). Therefore, it appears that HPB-ALL, as opposed to other cell lines, is not sensitive to changes in $\Delta\Psi_m$ induced by anti-CD95. On the contrary, the pro-apoptotic activity of quercetin might be mediated by the apoptotic pathway(s) correlated to reduction in $\Delta\Psi_m$.

3.7. Antioxidative effect of quercetin in HPB-ALL cells

Several observations suggest that reactive oxygen species (ROS) may mediate programmed cell death, although it is not clear how essential they are in generating apoptosis [37]. On the other hand, a role of antioxidants in enhancing apoptosis has been reported [38,39]. In addition, it has been demonstrated that an increase in superoxide anions blocks apoptosis triggered by CD95 in melanoma cells [40]. Therefore, we verified whether or not quercetin lowers ROS in our system. Fig. 7 shows that quercetin at a concentration of 50 μM was able to block ROS production in HPB-ALL cells, indicating that the molecule keeps its antioxidant property in the system we tested. The decrease in ROS production was detectable after 5 min of quercetin application to cells. We also demonstrated that: (i) ROS levels did not change in the presence of anti-CD95 antibody (data not reported); (ii) when quercetin and anti-CD95 were added together, ROS decreased with the same kinetic as shown in Fig. 7 (data not reported).

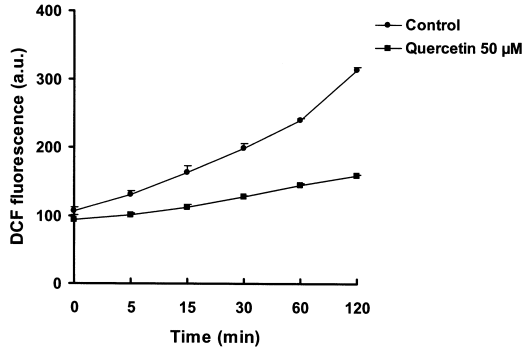

Fig. 7. Effect of quercetin on intracellular ROS. HPB-ALL cells (1×10^6) were treated with 10 μM DCFDA and incubated with 50 μM quercetin at different times. ROS production was monitored as DCF fluorescence as reported in Section 2. Each time point represents the average of two different experiments in duplicate ± standard error.

4. Discussion

In this report we have presented data on the pro-apoptotic effects of a naturally occurring flavonoid, quercetin, in a malignant cell line tested, namely HPB-ALL. We demonstrated that quercetin per se does not induce apoptosis. However, the molecule in association with anti-CD95 monoclonal antibody, active in inducing apoptosis in cells expressing CD95 receptor, strongly increases apoptosis. To our knowledge, this is the first time that the supposed pro-apoptotic activity of quercetin is linked to the CD95 pathway of apoptosis induction.

The concentration of quercetin used in this study is not far from that present in Mediterranean diet [6]. In rats, plasma concentration of quercetin reaches a value of 115 μM after an oral administration of 1 g/die [41], while in humans the plasma concentration of quercetin after a meal rich in flavonoids is in the micromolar range [42] to indicate a high uptake of the molecule. Finally, resveratrol, a well known cancer chemopreventive agent [43], is present in food at a concentration 10-fold lower than quercetin [7].

Quercetin is cytotoxic in HPB-ALL, where it causes mortality without arrest within 24 h. When we examined the rapid induction of apoptosis in HPB-ALL cells, we observed a positive effect of quercetin in enhancing CD95-induced apoptosis. It is worthwhile to note that the cell line considered in our study was resistant to CD95, and quercetin was able to bypass this resistance. Therefore, we will discuss possible mechanisms explaining quercetin activity.

A significant number of evidence correlates the antioxidant property of several molecules with their ability to induce apoptosis. *N*-acetyl-L-cysteine lowers ROS production and inhibits the mitogenic activity of cells expressing H-Ras^{V12} [39,44]. Similarly, the antioxidants PDTC (pyrrolidinedithiocarbamate) and vitamin E induce apoptosis in colorectal cancer cell lines [38]. Finally, CD95-mediated apoptosis is inhibited by increasing concentration of superoxide anion [40]. Therefore, we might hypothesize that quercetin increased apoptosis in our systems simply acting as antioxidant and lowering the level of ROS (Fig. 7). However, our preliminary data showed that this is not the case. In fact, different flavonoids, structurally similar to quercetin, are able to lower ROS production in HPB-ALL as reported for quercetin (Fig. 7), but the same molecules do not induce apoptosis in association with anti-CD95 (M. Russo and G.L. Russo, in preparation). In addition, the controversial theory that cell death is often associated with a strong production of ROS [45] has not been verified in our cells, according to our hypothesis that ROS are not involved in quercetin action. In fact, HPB-ALL cells treated with anti-CD95 do not show any variation of ROS levels (data not reported). Our results are in agreement with others suggesting that ROS production might be a consequence and not the cause of apoptosis [37,46], but differ from those reporting that ROS production is associated with anti-CD95 treatment [47]. Presently, we cannot exclude

that quercetin might modify key functional residues on proteins (e.g. sulphydryl groups) and thereby alter their function. Several examples of these mechanisms are reported in literature, e.g. [37].

The apoptotic signaling initiated by CD95 is transmitted to caspase-3 throughout two main pathways: the DISC/caspase-8 [26], and the mitochondria/cytochrome C/Apaf-1/caspase-9 pathways [27]. Mitochondria play an important role in cell death signaling [48]: alterations in mitochondrial structure and function occur in early stages of apoptosis [49]. In several systems, activation of CD95 to its receptors leads to alteration in mitochondrial membrane with the consequent release of cytochrome C that binds to Apaf-1 triggering apoptosis [48,50]. In addition, Scaffidi et al. [27] reported that in relation to the cell type two different pathways of CD95-mediated apoptosis can be identified: type I cells involve strong caspase-8 activation bypassing mitochondria, while type II cells hold an essential role due to the loss of mitochondria function. We believe that HPB-ALL cells belong to type I for the following reasons: (i) Fig. 6 of this report shows that anti-CD95 does not induce any variation in $\Delta\Psi_m$; (ii) immunoblotting of anti-CD95 treated HPB-ALL cells indicated that the level of Bcl-2 expression does not change (M. Russo and G.L. Russo, in preparation), while mitochondrial-induced apoptosis is blocked by overexpression of *bcl-2* [27] and down-regulation of *bcl-2* is associated with CD95-mediated apoptosis in several systems [51]; therefore, it is plausible that mitochondria are not involved. Concerning the mechanism explaining quercetin activity, it clearly involves the loss of mitochondrial function (Fig. 6); thus, it is reasonable that induction of cytochrome C by quercetin activates the apoptotic Apaf-1/caspase-9 pathway increasing the answer to anti-CD95 observed in our cells. It remains to be demonstrated whether quercetin triggers $\Delta\Psi_m$ directly or throughout the generation of another intermediate able to modify mitochondrial membrane potential. Accordingly, a recent work reports that quercetin is able to inhibit mitochondrial membrane permeability transition [52]. An apparent contradiction of our model is that quercetin by itself does not induce apoptosis. The puzzle can be solved by hypothesizing that the amount of cytochrome C released by quercetin is not sufficient to activate caspases, but remains docked by its high-affinity binding sites to electron transport chain until CD95 pathway is activated [48].

Unlike normal cells, many cancer cells are relatively resistant to CD95-mediated apoptosis. This advantage could be fundamental to tumor progression. Different mechanisms have been described to explain how alterations of the CD95 system could result in the escape of tumor cells from immunosurveillance [53]. Among cancer types relatively resistant to CD95-mediated cell death, are included: colon cancer and T-ALL from which the cell line used in the present study is derived [25]. One way to recover CD95 sensitivity is to employ chemotherapeutic agents such as quercetin that might enhance apoptosis in cancer cells by activating alternative pro-apoptotic pathways parallel to CD95 (as possibly in our case), or independent from it. In this respect, quercetin is currently in use in phase I clinical trials at doses not exceedingly different from the concentration generally used in the in vitro study and with low toxicity [54].

Considering the abundance of quercetin in Mediterranean diet, a better knowledge of its antineoplastic and pro-apoptotic properties could support its potential use as chemopreventive agent.

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