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Research paper

'In vitro' antioxidant and photoprotective properties and interaction with model membranes of three new quercetin esters

Antonella Saija[a,*], Antonio Tomaino[a], Domenico Trombetta[a], Maria Luisa Pellegrino[a], Beatrice Tita[b], Chiara Messina[c], Francesco P. Bonina[d], Concetta Rocco[e], Giovanni Nicolosi[e], Francesco Castelli[c]

[a]*Department Farmaco-Biologico, University of Messina, Messina, Italy*
[b]*Department of Pharmacology of Natural Substances and General Physiology, University of Rome 'La Sapienza', Rome, Italy*
[c]*Department of Chemical Sciences, University of Catania, Catania, Italy*
[d]*Department of Pharmaceutical Sciences, University of Catania, Catania, Italy*
[e]*Institute CNR for the Study of Natural Substances, Valverde, Italy*

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Abstract

Quercetin is well known to possess the strongest protective effect against UV light-induced lipoperoxidation. However, the absolute water insolubility of quercetin is a key step that may limit its bioavailability and, thus, its 'in vivo' employment as a photoprotective agent. The aim of the present paper was to evaluate 'in vitro' the antioxidant and photoprotective properties and the interaction with model membranes of three new semisynthetic quercetin derivatives, quercetin-3-*O*-acetate (Q-ac), quercetin-3-*O*-propionate (Q-pr) and quercetin-3-*O*-palmitate (Q-pal), obtained by esterification of the C-3 OH function with an aliphatic side-chain of different length. The antioxidant activity of quercetin and of its three esters was assessed in two 'in vitro' experimental models: (a) the bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical; (b) UV radiation-induced peroxidation in multilamellar vesicles (MLVs). Differential scanning calorimetry on dimyristoylphosphatidylcholine MLVs and unilamellar vesicles was employed to investigate the interaction of the drugs tested with model membranes. Finally, the stability following UV light exposure and the lipophilicity and water solubility of quercetin and its three esters were examined. The findings obtained demonstrated that the esterification with an opportune aliphatic side chain of the OH function located at the C-3 position allows the production of new quercetin derivatives, which may be good candidates as photoprotective agents. In particular, one could speculate that the esterification with a short side-chain (such as in Q-ac and Q-prop) provides the suitable chemico–physical features not only to maintain the antioxidant and photoprotective effectiveness of the parent drug, but also to be able to migrate through the aqueous environment and to interact with and cross phospholipid membranes.
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1. Introduction

In the last few decades the knowledge of the effects of ultraviolet (UV) radiation on human health, especially in some skin pathological conditions, but also in immunosuppression and eye damage, has grown strongly [1–5]. The range of UV spectrum consists of ultraviolet A (UVA; 320–400 nm), ultraviolet B (UVB; 280–320 nm) and ultraviolet C (UVC; 200–280 nm). However, the human population is exposed mainly to the dangerous effects of UVA and UVB radiation, since UVC arrives only partially to the earth and is absorbed by the superficial skin layers.

Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot}$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) are responsible, partially at least, for UV-induced deleterious effects. In fact, UVA is absorbed by all cellular constituents and induces mainly oxidative damage indirectly, whereas UVB induces mainly dipyrimidine photoproducts in DNA by a direct photochemical mechanism [1].

* Corresponding author. Department Farmaco-Biologico School of Pharmacy, University of Messina Contrada Annunziata, 98168 Messina, Italy. Tel.: +39-90-6766530; fax: +39-90-3533142.
E-mail address: saija@pharma.unime.it (A. Saija).

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The increasing solar UV radiation and changes in lifestyle strengthen the necessity to seek preventive measures effective against deleterious effects caused by intense or prolonged UV light exposure. The skin is the first region of the human body to be in contact with UV from sunlight and then other areas are presumably affected by circulation. Topical and systemic administration of natural or synthetic antioxidants represents a successful strategy for preventing the occurrence and for reducing the severity of UV-related deleterious effects [6–14].

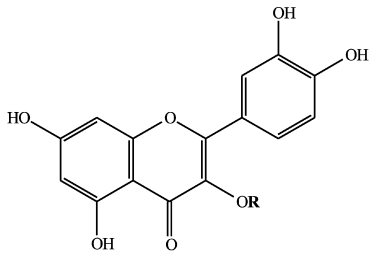
Flavonoids are phenolic compounds widely found in vegetable food; the average daily consumption of flavonoids in human diet is estimated to be about 1 g [15]. Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is a member of the flavonoid family; it is estimated that its intake with foods is 50–500 mg/die [16]. Flavonoids may delay oxidant injury and cell death by scavenging ROS and free radicals, protecting against lipid peroxidation and thereby terminating the chain-radical reaction, and chelating metal ions [17–18]. In particular, quercetin has been shown to scavenge $O_2^{\cdot}$, singlet oxygen ($^1O_2$) and ·OH radicals, to prevent lipid peroxidation, to inhibit cyclooxygenase and lipooxygenase enzymes, and to chelate transition metal ions [19,20].

Several authors have demonstrated the capability of quercetin in reducing the harmful effects of UV irradiation. Quercetin is able to protect 'in vitro' phosphatidylcholine (PC) multilamellar vesicles (MLVs) against UVC light-induced peroxidation [21] and skin-associated cell types from oxidative stress [22]. When administered intraperitoneally, quercetin is able to protect against liver oxidative damage in rats exposed to UVA radiation [9], and oral intake of quercetin prevents UV-induced damage in skin antioxidant systems in Sprague–Dawley rats [23] and UV-induced skin immunosuppression in SKH-1 hairless mice [24].

The biological properties of flavonoids are strictly related to their chemical structure and the choice of opportune structural features allows the optimization of biological activity, as well as of lipophilicity, water solubility and bioavailability. In particular, the absolute water insolubility of quercetin is a key step that may limit its bioavailability; for example, unlike other flavonoids such as naringenin and hesperetin, quercetin has a very poor capability to permeate through human skin [21]. Thus, quercetin cannot be a good candidate for a successful employment as a photoprotective agent.

For this reason, in the present paper we evaluated 'in vitro' the antioxidant and photoprotective properties and the interaction with model membranes of three new semisynthetic quercetin derivatives, quercetin-3-*O*-acetate (Q-ac), quercetin-3-*O*-propionate (Q-pr) and quercetin-3-*O*-palmitate (Q-pal), obtained by esterification of the C-3 OH function with an aliphatic side-chain of different length (Fig. 1).

The antioxidant activity of quercetin and of its three esters was assessed in two 'in vitro' experimental models,



Quercetin: R = H

Quercetin-3-acetate: R = —C(O)—CH_3

Quercetin-3-propionate: R = —C(O)—CH_2—CH_3

Quercetin-3-palmitate: R = —C(O)— CH_3

Fig. 1. Chemical structure of quercetin (**1**), quercetin-3-*O*-acetate (**2**; Q-ac), quercetin-3-*O*-propionate (**3**; Q-pr) and quercetin-3-*O*-palmitate (**4**; Q-pal).

which may also be rationally predictive for a successful employment of a drug as a photoprotective agent: (a) the bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·); and (b) UV radiation-induced peroxidation in liposomal membranes. Differential scanning calorimetry (DSC) on dimyristoylphosphatidylcholine (DMPC) MLVs and unilamellar vesicles (LUVs) was employed to investigate the interaction of the drugs tested with model membranes. This biomimetic experimental model was chosen because it can give information about drug capability to interact with phospholipid bilayers and to cross biological barriers. Finally, the stability following UV light exposure of quercetin and its three esters was examined along with their lipophilicity and water solubility.

2. Materials and methods

2.1. Materials

Synthetic L-α-dimyristoylphosphatidylcholine was obtained from Fluka Chemical Co. (Buchs, Switzerland); solutions of the lipid were chromatographically pure as assessed by two-dimensional thin-layer chromatography. The 1,1-diphenyl-2-picrylhydrazyl radical, egg yolk phosphatidylcholine, Tris–HCl, ethanol, methanol and phosphoric acid were purchased from Sigma-Aldrich srl (Milan, Italy). Tetrahydrofuran, trifluoroacetic acid and acetonitrile were purchased from Carlo Erba Reagenti (Milan, Italy). Quercetin was purchased from Extrasynthèse (Genay, France); the quercetin esters Q-ac, Q-pr and Q-pal were synthesized according to the method previously described by Lambusta et al. [25].

2.2. Quenching of DPPH· (DPPH· test)

The free radical-scavenging capacity of quercetin and of the quercetin esters Q-ac, Q-pr and Q-pal was tested as

bleaching of the stable radical DPPH· [26]. The reaction mixture (3.5 ml of methanol) contained 100 μM DPPH and different concentrations of quercetin or of a quercetin ester (0.5, 1, 2, 4, 6 μM); an equal volume (30 μl) of the solvent (methanol) employed to dissolve the compound tested was added to control tubes. After 20 min at room temperature, the absorbance was recorded at 517 nm. All experiments were carried out in duplicate and repeated at least three times. Results were expressed as percentual decrease with respect to control values; mean scavenging concentrations (SC_{50}) and 95% confidence limits (95% C.L.) were calculated by using the Litchfield and Wilcoxon test.

2.3. UVC radiation-induced peroxidation in liposomal membranes (UV-IP test)

The protective effect of quercetin and of the quercetin esters Q-ac, Q-pr and Q-pal against UVC-induced peroxidation was evaluated on PC MLVs by monitoring malondialdehyde (a final product of fatty acid degradation; MDA) production [27]. Briefly, 100 mg of PC, dissolved in chloroform, was transferred to a small stoppered tube. The lipid was thoroughly dried under nitrogen. It was then dissolved in warm ethanol (80 mg), and 25 mM Tris–HCl, pH 7.4 (200 mg) was added to yield a (100:80:200, by wt.) lipid/ethanol/water mixture. This mixture was heated to 60 °C for a few minutes and then allowed to cool to room temperature (20 °C) yielding a proliposome mixture. The proliposome mixture was finally converted to a liposome suspension by the dropwise addition of 25 mM Tris–HCl, pH 7.4, to a final volume of 10 ml. The suspension was vortex-mixed throughout this last stage. Liposome dispersion (1 ml in a glass cylindrical flask with a 3 cm^2 exposure surface area and without stopper) was maintained at room temperature and exposed, for 1.5 h, to UV-radiation from a 15-W Philips germicidal lamp (254 nm) at a distance of 10 cm; the dose rate of UV-radiation was 105 erg/mm^2 per s. Different concentrations of quercetin (1, 2, 4, 6, 8 μM) or of a quercetin ester (1, 2, 4, 6, 8, 12, 16, 20, 24 μM) were added to the system; an equal volume (50 μl) of the vehicle alone (ethanol) was added to control tubes. MDA concentration in the mixture was measured by using a colorimetric assay kit (Calbiochem-Novabiochem Corp., La Jolla, CA, USA). All experiments were carried out in triplicate and repeated at least three times. Results were expressed as percentual decrease with respect to control values; mean inhibitory concentrations (IC_{50}) and 95% confidence limits (95% C.L.) were calculated by using the Litchfield and Wilcoxon test.

2.4. Stability following UV light exposure

To test their stability following UV light exposure, a water/ethanol (95:5, v/v) solution of quercetin or of a quercetin ester (18 μM) was exposed to UVC radiation under the same experimental conditions described above and the spectra were recorded at different times (0, 30, 60, 90 min) from the beginning of the incubation [21].

2.5. Differential scanning calorimetry (DSC)

Experiments were carried out according to a previously-described method [28]. Dimyristoylphosphatidylcholine (DMPC) MLVs were prepared in the presence and absence of quercetin or of quercetin esters by the following procedure: chloroform-methanol (1:1, v/v) stock solutions of DMPC and one of the compounds under investigation (1:1, v/v) were mixed to obtain the chosen mole fractions (X_D). The solvents were removed under a nitrogen flow in a rotoevaporator, and the resulting film was freeze-dried under vacuum to remove the residual solvents. Liposomes were obtained by adding 50 mM Tris buffer (pH 7.4) to the film, then heating at a temperature (37 °C) above that of the gel–liquid crystalline phase transition and vortexing three times for 1 min. The samples were shaken for 1 h in a water bath at 37 °C to homogenize the liposomes.

Aliquots of 120 μl of lipid aqueous dispersion (5 mg of lipid), pure or containing the drugs under investigation, were transferred to 150 μl DSC aluminum, hermetically sealed, pan (Mettler Toledo Group, Greifensee, Switzerland) and submitted to DSC analysis. The temperature of the maximum of the transition endotherm peak (T_m) and the enthalpy changes (ΔH) were determined by using a Mettler TA 4000 system equipped with a DSC-30 cell and a TC-11 processor (Mettler Toledo Group). The scan rate employed was 2 °C/min in a temperature range of 2–37 °C. The sensitivity was 1.72 mW, and the reference pan was filled with Tris buffer solution. ΔH values were evaluated from the peak areas using the integration program of the TA processor, permitting the choice of different baselines and ranges of integration. For curves showing an ill-defined baseline, a fixed arm planimeter was also employed. The areas calculated with these different methods lie within the experimental error (±5%). After calorimetric scans, all samples were extracted from the pan and aliquots were used to determine the amount of phospholipid by the phosphorous assay [29]. Data were expressed as mean ± S.D. of at least five calorimetric scans and statistically analyzed by the Student's *t*-test for unpaired data; statistical significance was accepted when $P < 0.05$.

Furthermore, DSC analysis allows us to obtain evidence of membrane penetration by comparing the thermotropic effects exerted by a compound put in contact with empty LUVs and then following the reaching of the maximum perturbative effect on the two layered membranes [30]; if the perturbative effect (T_m shift) of the two systems tends to the same value, which is comparable with that observed when the compounds have been completely dispersed in the bilayer during the liposomal preparation, this should represent a signal of the happened membrane penetration. Thus, to study the capacity of quercetin and of its esters to permeate the model membrane, a kinetic experiment was

performed by putting a suspension of empty DMPC LUVs in contact with a fixed amount of the powdered drug (0.12 M fraction) placed in the bottom of the DSC crucible [26,29–30]. The empty LUVs were prepared by submitting empty MLVs to extrusion through polycarbonate membranes of 100 nm (Avestin Inc., Ottawa, Canada) in an extruder system (LiposoFast™ Basic, Avestin Inc.) [31]. The crucible was gently shaken for 10 s and submitted to the following heating, isothermal and cooling calorimetric cycles: (1) a first scan between 2 and 37 °C, to detect the interaction between the compound and model membrane; (2) an isothermal period of 1 h at 37 °C to permit the drug tested to permeate (if able) the lipid layer(s) in a disordered state at a temperature above the lipid transitional temperature; (3) a cooling scan between 37 and 2 °C to restart the heating program. This procedure was run at least five times.

2.6. Log capacity factor (log K')

Reverse-phase chromatographic retention times can be used to estimate oil/water partition coefficients; a good correlation is found between log octanol/water partition coefficients and log K' using octadecyl silica columns [21]. Log K' values for quercetin and the three esters, Q-ac, Q-pr and Q-pal were determined by HPLC with diode array detection. The HPLC apparatus consisted of a liquid chromatograph LC-10ADvp (Shimadzu, Kyoto, Japan), equipped with a SPD-M10Avp diode array detector (Shimadzu). Integration of the chromatographic peaks was achieved with a Class-VP5 software (Shimadzu). Chromatography was performed on a Prodigy 5 μ ODS-3 100 A column (250 × 4.60 mm, particle size: 5 μm; Phenomenex, Macclesfield, UK). The mobile phase was water/acetonitrile/tetrahydrofuran/trifluoroacetic acid (58.8:40:1.2:0.06). The flow-rate was set at 1.0 ml/min.

Each compound was dissolved in methanol to give a final concentration of 10 μg/ml; samples were filtered prior to injection using a Millex HV13 filter (0.22 μm, Waters-Millipore Corporation, Milford, MA, USA) and an aliquot (20 μl) was injected into the HPLC apparatus. Detection was effected at 254 nm.

Log K' values were calculated from the following relationship:

$$\log K' = \log \frac{T_r - T_0}{T_0}$$

where T_r is the retention time of the flavonoid peak and T_0 denotes the retention time of the non-retained solvent peak.

2.7. Water solubility

The water solubility of quercetin and of the three esters Q-ac, Q-pr and Q-pal was determined according to the method described by Bonina et al. [32]. An excess amount of each compound was weighed into a glass tube containing 2 ml of water; the tube was sealed with a teflon lined cap. The mixture was stirred by a magnetic stirrer for 24 h at room temperature; therefore, it was filtered using a Millex HV13 filter (0.22 μm, Waters-Millipore Corporation) and the concentrations of the compounds in their saturated solutions were determined by the HPLC method described above.

3. Results and discussion

As demonstrated by results obtained in the DPPH· test, all drugs tested (quercetin and the quercetin esters Q-ac, Q-pr and Q-pal) elicit a good concentration-dependent scavenging effect, allowing the calculation of a SC_{50} (see Table 1). In particular it is evident that the esterification of the OH function located at the C-3 position with different aliphatic side chains does not mask the scavenging activity of the parent drug quercetin.

A brief methodological comment is needed before discussing the results obtained in the UV-IP test. Biologically relevant solar radiation is represented mainly by UVB and, to some extent, UVA wavelengths. However, each region of the UV spectrum can induce strong degradation of fatty acids (the main components of biological membranes) by means of the same mechanism. High energy UVC radiation is widely employed in 'in vitro' cell-free systems (particularly liposomal systems), where production of MDA is monitored as an index of lipid peroxidation; in fact in these experimental systems, higher MDA levels may be expected following UVC exposure than after UVB or UVA irradiation.

As shown in Table 1, quercetin appeared able to protect PC within liposomal bilayers from UVC-induced peroxidation. In fact, exposure of PC liposomes to UV radiation for 1.5 h elicited a large increase in MDA production (69.58 ± 0.83 μmol/ml); the addition of quercetin reduced the amount of formed MDA in a dose-dependent manner (IC_{50} 5.62 μM). Similar results were obtained when Q-ac or Q-pr were added to the experimental system. However, Q-pal appeared really less effective than quercetin, Q-ac and

Table 1
Scavenging effect against the stable radical DPPH· and inhibition of UVC light-induced peroxidation on PC MLVs in the presence of different concentrations of quercetin, Q-ac, Q-pr and Q-pal

Drug	DPPH test SC_{50}[a] (95% C.L.)[b] (μM)	UV-IP test IC_{50}[c] (95% C.L.)[b] (μM)
Quercetin	4.42 (4.23–4.64)	5.62 (5.19–6.09)
Quercetin-3-O-acetate	4.35 (4.14–4.57)	5.17 (4.88–5.51)
Quercetin-3-O-propionate	3.18 (2.90–3.52)	5.21 (4.82–5.67)
Quercetin-3-O-palmitate	3.92 (3.71–4.13)	16.66 (15.71–17.84)

Experiments were carried out as described in Materials and methods.
[a] Mean scavenging concentrations.
[b] 95% confidence limits.
[c] Mean inhibitory concentrations.

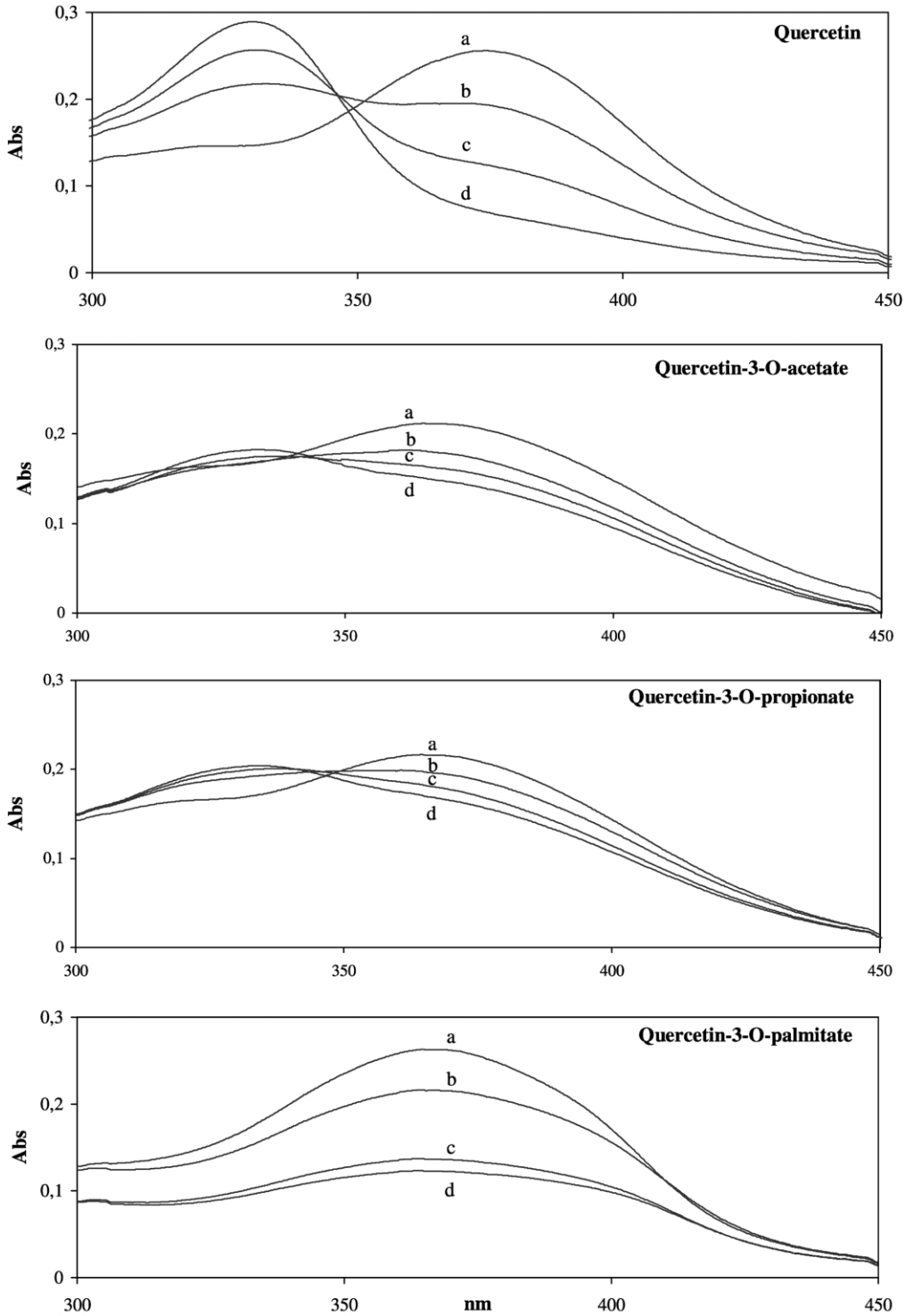


Fig. 2. Spectral changes of a water/ethanol (95:5, v/v) solution of 18 μM quercetin, Q-ac, Q-pr and Q-pal, exposed to UV irradiation. The spectra were registered at different times (a = 0 min; b = 30 min; c = 60 min; d = 90 min) from the beginning of the incubation.

Q-prop; in fact a significantly higher IC_{50} (16.66 μM) was calculated for this ester. Since there is considerable evidence relating radical oxygen species with UV light-induced phospholipid degradation, the photoprotective effect of quercetin and of its esters tested might be due to its capability to scavenge UV-induced aqueous and lipophilic free radicals and, thus, to inhibit propagation of lipid peroxidative chain reaction.

Aiming at better clarifying the photoprotective action of the drugs under investigation, we exposed a 18 μM solution

Table 2
Effect of quercetin, Q-ac, Q-pr and Q-pal, at different molar fractions, on the thermodynamic parameters of aqueous dispersion of DMPC MLVs

Molar fraction	Quercetin			Q-ac			Q-pr			Q-pal		
	T_m (°C)	$(\Delta T/T°_m) \times 10^3$	ΔH (J/g)	T_m (°C)	$(\Delta T/T°_m) \times 10^3$	ΔH (J/g)	T_m (°C)	$(\Delta T/T°_m) \times 10^3$	ΔH (J/g)	T_m (°C)	$(\Delta T/T°_m) \times 10^3$	ΔH (J/g)
0.00	24.0 ± 0.11	0.0	34.5 ± 1.1	24.0 ± 0.11	0.0	34.5 ± 1.1	24.0 ± 0.11	0.0	34.5 ± 1.1	24.0 ± 0.11	0.0	34.5 ± 1.1
0.015	23.8 ± 0.14	0.67	29.3 ± 0.9	23.6 ± 0.15	1.3	28.5 ± 1.0	23.6 ± 0.13	1.5	34.1 ± 1.2	23.8 ± 0.15	0.7	34.9 ± 1.3
0.030	23.3 ± 0.18	2.36	24.4 ± 0.8	23.4 ± 0.17	2.0	23.2 ± 0.8	23.3 ± 0.17	2.3	29.2 ± 1.5	23.6 ± 0.14	1.3	32.0 ± 1.1
0.045	22.8 ± 0.17	4.04	21.1 ± 0.8	22.6 ± 0.14	4.7	19.6 ± 0.9	22.7 ± 0.14	4.4	27.0 ± 1.3	23.5 ± 0.18	1.7	30.5 ± 1.5
0.060	21.5 ± 0.15	8.45	4.7 ± 0.3	21.7 ± 0.16	7.7	3.8 ± 0.5	22.2 ± 0.17	6.2	33.8 ± 1.5	22.8 ± 0.21	3.9	30.8 ± 1.2
0.090	18.7 ± 0.16	17.8	2.1 ± 0.3	19.5 ± 0.13	15.1	2.0 ± 0.3	19.5 ± 0.12	15.1	4.5 ± 1.2	21.5 ± 0.19	8.4	21.3 ± 0.9
0.120	17.3 ± 0.19	22.6	1.8 ± 0.09	18.3 ± 0.18	19.1	1.5 ± 0.06	17.8 ± 0.15	20.9	3.8 ± 0.09	19.8 ± 0.23	14.1	20.3 ± 1.1

Data are expressed as mean ± S.D. of at least five calorimetric scans.

of quercetin, Q-ac, Q-pr or Q-pal to UVC irradiation and recorded the spectra at different times (0, 30, 60, 90 min) from the beginning of the incubation. Exposure of quercetin, Q-ac and Q-pr to UVC radiation caused marked changes in its absorption spectra, characterized by the presence of an excellent isosbestic point, that is indicative of new product formation (Fig. 2). In relation to Q-pal, only a significant decrease in maximum absorbance was observed during the experimental period (Fig. 2). These spectral modifications lead us to hypothesize that quercetin, Q-ac and Q-pr might provide their protective effect against UVC radiation-induced damage also by acting as a UV-absorbing screen. Conversely, Q-pal does not appear to be a chemical species (or to produce a chemical species) stable when exposed to UVC irradiation, and thus cannot be considered a promising candidate for employment as photoprotective agent.

Thus, in order to better investigate the capability of these compounds to interact with and cross biological membranes, we investigated, by DSC, the potential capability of quercetin and of its three esters to interact with phospholipid bilayers. In fact, DSC is a non-perturbative thermodynamic tool, which permits us to investigate the effect exerted by a drug on the phase transition gel-to-liquid crystal of a phospholipid species, and to study the uptake process of a solid compound on a model membrane surface [30].

The first series of experiments was carried out on DMPC MLVs. Table 2 reports the effect of quercetin, Q-ac, Q-pr and Q-pal, at increasing drug molar fractions, on two thermodynamic parameters of DMPC MLVs, the transitional temperature (T_m) and ΔH (a parameter related to the peak area).

In this table the shift of T_m values is expressed also as $(\Delta T/T^0_m)10^3$, where $\Delta T = T_m - T^0_m$, T^0_m is the transition

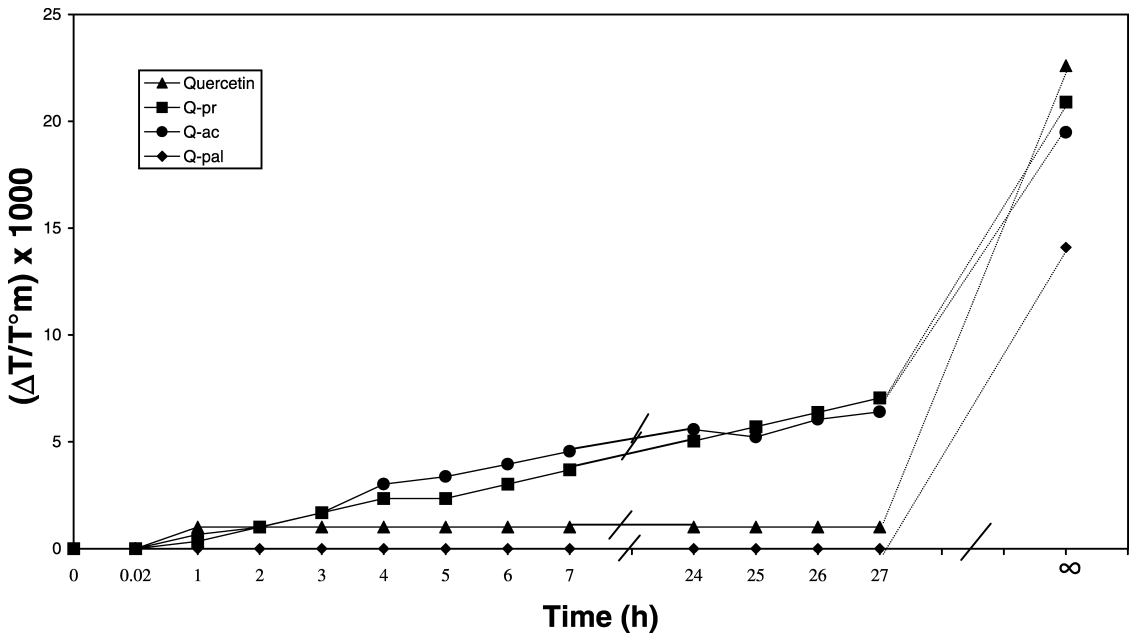


Fig. 3. Transitional temperature variations, expressed as $(\Delta T/T^0_m) \times 10^3$, of DMPC LUVs in the presence of 0.12 M ratio of quercetin, Q-ac, Q-pr and Q-pal for increasing incubation time. The value of time infinite refers to samples prepared for direct interaction between quercetin or its derivatives and MLVs, by organic solvent dissolution and MLV preparation; these values are to be considered as the maximum interaction between compounds and vesicles.

temperature of pure DMPC MLVs and T_m is the transition temperature obtained at increasing molar fractions of the compound present in the lipid aqueous dispersion. The results of these experiments have evidenced a good capability of all drugs tested to interact with phospholipid bilayers. In fact, a statistically significant shift of T_m values associated to the gel to liquid-crystal phase transition toward lower temperatures was observed at increasing drug molar fractions. The efficiency order was: quercetin > Q-pr > Q-ac > Q-palm. These changes in T_m values are very likely due to the introduction of lipophilic molecules into the ordered structure of the lipidic bilayer. It is well known that drug molecules can act as spacers in such a structure, causing a destabilization of the lipid mosaic with a decrease in the T_m of the gel to liquid-crystal phase transition [33–35]. Furthermore, it is evident that the T_m shift elicited by the quercetin esters appears greater for the short side-chain substituted drugs (Q-ac and Q-pr) than for Q-pal.

Concerning the **ΔH** values, quercetin, Q-ac, Q-pr and Q-pal appeared able to induce a significant modification in this thermodynamic parameter, which is indicative of a change in the cooperativity of the phospholipid acyl chains in the lipid bilayer.

In the second part of DSC experiments, kinetic measures were carried out to investigate the capability of the drugs under study to migrate through the aqueous medium and permeate the LUV membrane. The results obtained (shown in Fig. 3) are due to a succession of states consisting of the drug passing from the solid to the solution state, crossing the aqueous medium and then reaching, penetrating into and remaining in the lipidic membrane. The employment of LUVs, instead of MLVs, allows a faster observation of the interaction between the compounds examined and the model membrane, without waiting for the drugs to cross concentric bilayers of DMPC MLVs. To obtain evidence of membrane penetration, we compared the maximum perturbative effect exerted by each compound put in contact with empty LUVs (molar fraction = 0.120) with those obtained when the drug was completely dispersed in the bilayer during the liposomal preparation (so that it is compelled to penetrate and stay inside liposomes). If in the kinetic curves the perturbative effect (T_m shift) of the two systems tends to the same value (dashed curves), this should represent a signal of the membrane penetration.

Curves reported in Fig. 3 show that only Q-ac and Q-pr (but not quercetin and Q-pal) appeared able to migrate through the aqueous environment and to interact with phospholipid bilayers, although they do not reach the maximum of the interaction represented by the phase organic preparation (interaction at infinite time).

Finally, as shown in Table 3, log K' calculated for quercetin, Q-ac, Q-pr and Q-pal are 0.250, 0.318, 0.594 and 0.947, respectively; thus, longer is the side chain of the ester, higher the lipophilicity of the drug. Furthermore, the order of water solubility for the four compounds examined was: Q-ac >> Q-pr > quercetin > Q-pal (Table 3); this means that esterification of the C-3 OH function with a short aliphatic chain (1–3 C) significantly increases the aqueous solubility of the parent drug quercetin.

Table 3
Lipophilic indices (log K') and water solubility of quercetin, Q-ac, Q-pr and Q-pal

Drug	Parameters	
	Log K'	Water solubility (μg/ml)
Quercetin	0.250	0.514
Quercetin-3-*O*-acetate	0.318	240.325
Quercetin-3-*O*-propionate	0.594	6.384
Quercetin-3-*O*-palmitate	0.947	N.D.

Experiments were carried out as described in Materials and Methods. N.D., not determinable.

The presence of different substituents in the backbone structure is well known to modulate the interaction of a drug with membranes, as well as their physicochemical properties. In this study, lipophilicity does not appear to be the key parameter in determining drug permeation through bilayers. In fact, the best interaction with phospholipid bilayer was observed for quercetin, that is the less lipophilic among the compounds tested (see Table 2). Moreover, in the kinetic experiments, only the drugs with a discrete water solubility, Q-ac and Q-pr, appeared able to reach phospholipid vesicles.

In conclusion, the present findings demonstrate that the esterification with an opportune aliphatic side chain of the OH function located at C-3 position allows the production of new quercetin derivatives, which may be good candidates as photoprotective agents. In particular, one could speculate that the esterification with a short side-chain (such as in Q-ac and Q-prop) provides the suitable chemico–physical features not only to maintain the antioxidant and photoprotective effectiveness of the parent drug, but also to be able to migrate through the aqueous environment and to interact with and cross phospholipid membranes.

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