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Inhibition of Oxidant-Induced Lipid Peroxidation in Cultured Renal Tubular Epithelial Cells (LLC-PK_1) by Quercetin

M.K. KUHLMANN*, G. BURKHARDT, E. HORSCH, M. WAGNER and H. KÖHLER

Universitätskliniken des Saarlandes, Innere Medizin IV, Nephrologie, D-66421 Homburg/Saar, Germany

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The protective effect of quercetin against oxidant-induced cell injury (hypoxanthine/xanthine oxidase system) was studied in the renal tubular epithelial cell line LLC-PK_1. Pretreatment with quercetin provided protection from structural and functional cell damage in a concentration-dependent manner (10–100 μM). Comparison with structural variants revealed that the protective property of quercetin depends on the number of hydroxyl substituents in the B-ring, the presence of an extended C-ring chromophore, 3-D-planarity and lipophilicity, indicating that membrane affinity is essential for protection. The hypothesis that quercetin exerts its protective effects via inhibition of lipid peroxidation was further examined. Protection by quercetin was found when lipid peroxidation, assessed by the release of malondialdehyde, was initiated by H_2O_2 or by the combination of 1-chloro-2,4-dinitrobenzene and aminotriazole. In contrast, the bioflavonoid was not protective when oxidative cell damage was induced by menadione and occurred in the absence of lipid peroxidation. These data suggest that cytoprotective effects of quercetin are related to membrane affinity and may be explained by interruption of membrane lipid peroxidation rather than by intracellular scavenging of oxygen free radicals.

Keywords: Bioflavonoids, quercetin, oxidative injury, protection, renal tubular cells, lipid peroxidation

INTRODUCTION

Bioflavonoids are a group of naturally occurring phenolic compounds with a wide range of biological activities.[1,2] Recently much attention has been paid to their antioxidant properties and their potency to inhibit lipid peroxidation, both related to their radical scavenging capacity.[3] The effects of flavonoids on free-radical-mediated processes have been studied *in vitro* and *in vivo*,[4] and there is growing evidence that antioxidant flavonoids might induce important biological effects in humans.[5] A recently published epidemiologic study suggests an inverse relationship between dietary flavonoid (especially quercetin)

* Corresponding author. Tel.: ++49-6841-16-3503. Fax: ++49-6841-16-3540.

consumption and risk of coronary heart disease.[6] The radical scavenging properties render bioflavonoids potentially useful for prevention and treatment of acute or chronic oxidative injury.

There is increasing evidence for an important role of oxygen free radicals in the pathogenesis of renal ischemia-reperfusion injury.[7–11] Since proximal tubules are highly aerobic they are important sites of injury in the ischemic kidney.[12] Here we report on protective effects of the bioflavonoid quercetin against oxidative injury in cultured renal tubular epithelial cells (LLC-PK_1) and on our results of structure–activity studies to further explore the potential mechanism of cytoprotection.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma Chemical Co. (Munich, FRG) unless otherwise noted.

Cell Culture

LLC-PK_1 cells (passage 196), an analog of proximal tubular cells, were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) and grown in phenol-red-free medium 199 (M 199) supplemented with 10% fetal calf serum (FCS; PAA Laboratories, Cölbe, FRG) and 4 mM L-glutamine (Greiner, Frickenhausen, FRG) at 37°C under a humidified atmosphere of 95% air and 5% CO_2 without addition of antibiotics. For experimental studies cells (passages 196–206) were grown to confluency in 96-, 24-, or 6-well plates and studied in a Hepes buffered salt solution, pH 7.4 (medium A; concentration in mM: NaCl 110.0, KCl 5.0, $MgSO_4$ 1.0, $KHCO_3$ 1.58, $CaCl_2$ 1.25, $NaHCO_3$ 6.42, Na_2HPO_4 1.0, Hepes 20.0, Glucose 5.55). Only confluent cell monolayers showing dome formation were used for experiments.

Induction of Oxidant Injury to Renal Tubular Epithelial Cells in Culture

Extracellular Oxidant-Generating systems

Hypoxanthine/xanthine oxidase model LLC-PK_1 cells grown in 96-well microtiter plates were exposed to 200 µl of a hypoxanthine/xanthine oxidase (HX/XO) reaction mixture (containing 500 µM hypoxanthine, 10 mU/ml xanthine oxidase, 75 µM EDTA, and 50 µM $FeCl_3$) in medium A for 60 min at 37°C. This reaction mixture generates superoxide, H_2O_2 and hydroxyl radical.[13] After 60 min of exposure, the HX/XO reaction mixture was replaced by 200 µl of FCS-free M199. Cell injury was measured 15 h after removal of the HX/XO reaction mixture, except for studies examining the time course of cell injury, where the time point for evaluation of cytotoxicity was varied between 3 and 21 h.

H_2O_2 model Cells were exposed to 1.5 mM reagent grade H_2O_2 in medium A supplemented with 50 µM $FeCl_3$ and 75 µM EDTA for 180 min and cell viability was studied immediately thereafter.

Intracellular Oxidant-Generating Systems

Cells were exposed to oxidative stress induced either by 100 µM 1-chloro-2,4-dinitrobenzene and 10 mM aminotriazole (CDNB + AT) for 4 h or by incubation with the quinone menadione (2.0 mM) for 3 h. CDNB stock solution (100 mM) was prepared in dimethylsulfoxide (DMSO) and AT stock solution in H_2O. Final DMSO concentration (0.1%) did not affect cell viability or cytotoxicity in the various models. Menadione was dissolved in medium A supplemented with 50 µM $FeCl_3$ and 75 µM EDTA. As described by others,[14] total cellular LDH activity was lowered on average by 30% through menadione treatment. Cell viability and lipid peroxidation were assessed immediately following incubation.

Bioflavonoid Studies

Flavonoid stock solutions (100 mM) were prepared in DMSO and stored in the dark at room temperature for up to 3 weeks. Direct interaction of antioxidants with H_2O_2-generating systems in the medium was avoided by removal of bioflavonoids prior to start of oxidant exposure. Cells were incubated with bioflavonoids for 3 h in FCS supplemented, phenol-red-free M199 (maximum final DMSO concentration: 0.2%). The bioflavonoid containing medium was replaced by medium A. Vehicle alone (DMSO) had no influence on cell viability. In some experiments diphenyl-*p*-phenylenediamine (DPPD), an inhibitor of lipid peroxidation, was employed. The DPPD stock solution (24.0 mM) was prepared in 93% ethanol. DPPD was added to FCS supplemented, phenol-red-free M199 (maximum final ethanol concentration: 0.6%).

Determination of Cell Injury

Lactate Dehydrogenase Release

The release of cytoplasmic lactate dehydrogenase (LDH) into the supernatant was used as indicator of structural cell damage. LDH activity was measured spectrophotometrically using a commercial cytotoxicity detection kit (Boehringer Mannheim, Mannheim, FRG).

MTT-Assay

Cell viability was assessed using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay[15] which was performed according to Mosmann.[16] As described by others,[17] data are expressed as percentage of untreated control cells.

Assessment of Lipid Peroxidation

The formation of malondialdehyde was measured in the supernatant as thiobarbituratic acid reactive substance (TBARS) according to Aust.[18] Supernatant (200 µl) was removed and butylated hydroxytoluene (final conc. 0.01%) was added immediately to the sample to minimize lipid peroxidation during processing. Spectrophotometric analysis was performed at 532 nm and the amount of TBARS (nmoles/ml) was derived from a standard curve generated by addition of increasing amounts of malondialdehyde (0.01–10 nmoles/ml) to medium A. TBARS was related to protein content of each well, measured according to Bradford.[19]

Statistical Analysis

Data are expressed as mean ± SD of at least three separate experiments with $n = 4–6$ each. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post-test for multiple comparisons.

RESULTS

Oxidant-Induced Injury to Cultured Renal Epithelial Cells

For studies on oxidant injury in proximal tubular epithelial cells a frequently employed model of extracellular enzymatic oxygen radical generation (hypoxanthine/xanthine oxidase [HX/XO] in the presence of EDTA-chelated iron) was used. This reaction mixture generates superoxide, H_2O_2 and hydroxyl radicals.[11,13,20] After 60 min of exposure the incubation medium was replaced by normal growth medium in the absence of FCS and cell injury was evaluated repeatedly during the following 21 h (Figure 1). Disturbances of membrane integrity and cell viability as determined by LDH release and MTT-test, respectively, were gradually increasing over time. Half maximal injury was observed at 8 h, when metabolic activity was reduced to 50.0 ± 3.1% and LDH release increased to 62.4 ± 4.3% of total cellular content. Maximum cell injury was observed at 19 h (MTT-test: 12.4 ± 4.2%, LDH-release: 77.3 ± 15.4%). In subsequent experiments, cell damage was assessed at 16 h after HX/XO exposure.

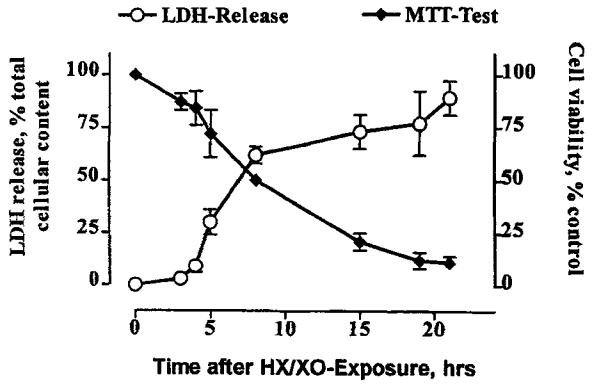


FIGURE 1 Oxidant-induced injury in LLC-PK_1 cells. Time course of cell injury produced by exposure of LLC-PK_1 monolayers to 500 μM hypoxanthine, 50 mU/ml xanthine oxidase, 75 μM EDTA, and 50 μM $FeCl_3$ for 60 min. Membrane damage is evaluated by LDH release (open symbols, left ordinate), cell viability is assessed by MTT-assay (filled symbols, right ordinate). Each time point represents mean ± SD of $n = 6$.

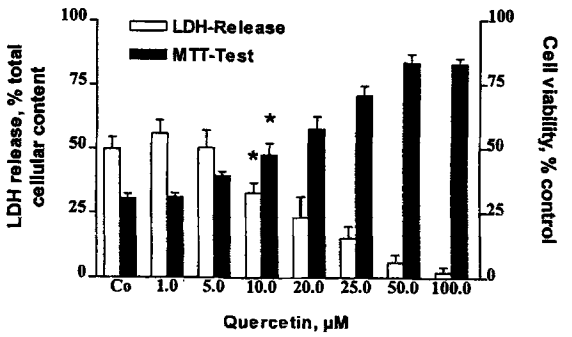


FIGURE 2 Protection from oxidant-induced injury by quercetin. Effects of various concentrations of quercetin on oxidant-induced injury in LLC-PK_1 cells measured 16 h after exposure to hypoxanthine (500 μM)/xanthine oxidase (50 mU/ml) for 60 min. Cell damage is evaluated by LDH release (open bars, left ordinate) and MTT-assay (black bars, right ordinate). Each condition represents mean ± SD of $n = 12$. Data were analyzed by ANOVA and each condition was compared to vehicle-treated controls (Co) by Dunnet's test. $^*p < 0.05$ for the lowest quercetin concentration providing significant protection vs. control cells.

Effect of Quercetin on Hypoxanthine/ Xanthine Oxidase-Induced Cell Injury

In preliminary studies we had observed that pretreatment of LLC-PK_1 cells with quercetin prevented oxidative injury. First, the possibility of extracellular radical scavenging through release of quercetin from pretreated cells into the HX/XO reaction mixture was excluded. Therefore, cells were incubated with either quercetin (100 μM) or vehicle (DMSO, 0.1%) for 3 h followed by replacement of the growth medium by plain medium A for 60 min, thus allowing for potential redistribution of quercetin into the supernatant. Then, the supernatant of quercetin-pretreated wells was cross-exchanged with supernatant of vehicle-treated wells, followed by addition of the HX/XO reaction mixture to each well for another 60 min. Cell injury was assessed 16 h later. It was our hypothesis that if protection was due to extracellular radical scavenging by quercetin redistributed from the cells into the medium, then, cells initially pretreated with vehicle and now exposed to supernatant from quercetin-pretreated cells should be protected from subsequent oxidant injury. This was not the case (LDH release: 62.0 ± 8.0% in controls, 1.4 ± 1.3% in quercetin-pretreated cells exposed to HX/XO in supernatant of vehicle-pretreated cells [$p < 0.001$ vs. control, $n = 12$] and 53.7 ± 6.5% in vehicle-pretreated cells exposed to HX/XO in supernatant of quercetin-pretreated cells, [p = n.s. vs. control, $n = 12$]), and it was concluded that extracellular radical scavenging did not explain protection.

Figure 2 illustrates the concentration dependence of the effects of quercetin on HX/XO-induced oxidative injury in LLC-PK_1 cells. Cells were pretreated with increasing concentrations of quercetin for 3 h followed by a 60 min HX/XO exposure and oxidative injury was assessed 16 h later. Quercetin was removed from the wells prior to HX/XO exposure. Quercetin concentrations ≥ 10 μM dose dependently reduced cell injury, whereas concentrations < 10 μM were not protective. LDH release of vehicle-pretreated cells was 49.8 ± 1.4% compared to 32.7 ± 1.2% for quercetin-pretreated (10 μM) cells. Similarly, the rate of MTT conversion increased from 30.5 ± 1.7% in controls to 47.5 ± 4.6% ($p < 0.05$) after pretreatment with 10 μM

quercetin. Protection as well as preservation of cell viability was complete at 50 μM (LDH release 5.9 ± 0.9%, MTT-test 83.6 ± 3.3%) and did not further increase with higher quercetin concentrations.

Structure–Activity Studies (Table I, Figure 3)

To further analyze the mechanisms of cytoprotection, structural variants of quercetin were examined. The compounds were selected in an attempt to allow for differentiation between antioxidative activity, molecular size, lipophilicity and 3-D structural characteristics and were tested at a fixed concentration of 100 μM each (Table I). LDH release was lowered from 57.0 ± 4.5% in vehicle-pretreated cells to 12.2 ± 3.1% ($p < 0.01$) in quercetin-pretreated cells and cell viability was almost completely preserved (MTT-test: 12.2 ± 3.9% vs. 95.1 ± 1.8% after quercetin pretreatment, $p < 0.01$). Kaempferol (LDH release 23.5 ± 3.6%, MTT-test 19.3 ± 1.7%) and apigenin (33.7 ± 4.7% and 24.6 ± 2.3%, resp.), which differ from quercetin by only one or two hydroxyl groups, respectively, were significantly less protective than quercetin. A variation in the position of the B-ring hydroxyl groups lowered protection (morin: 45.1 ± 0.9% and 26.7 ± 2.3%, resp.), as did the presence of a third B-ring hydroxyl group (myricetin: 56.8 ± 1.0% and 45.8 ± 1.4%, resp.). Interestingly, myricetin did not offer structural protection, although functional viability of oxygen radical exposed cells was preserved to some degree. Flavonoids lacking a 3-OH group (e.g. apigenin) or flavonoids bearing sterically hindering B-ring substitutents (e.g. morin) lose structural planarity of the AC-B-ring system accompanied by notable losses in electron conjugation and thus radical scavenging activity.[21] Furthermore, reduction of the C-ring double bond (naringenin: 49.8 ± 2.3% and 3.0 ± 2.0%, resp.) and the lack of the extended C-ring chromophore (catechin: 60.7 ± 1.0% and 14.3 ± 0.9%, resp.), both totally abolished protection. Silibinin, a bioflavonoid significantly larger than quercetin, was not protective (46.7 ± 1.0% and 22.8 ± 1.4%, resp.). It is important to note that glycosylation of the otherwise intact quercetin structure (rutin: 51.7 ± 2.1% and 8.2 ± 0.7%, resp.) which leads to a reduction in lipophilicity, completely abolished cytoprotection.

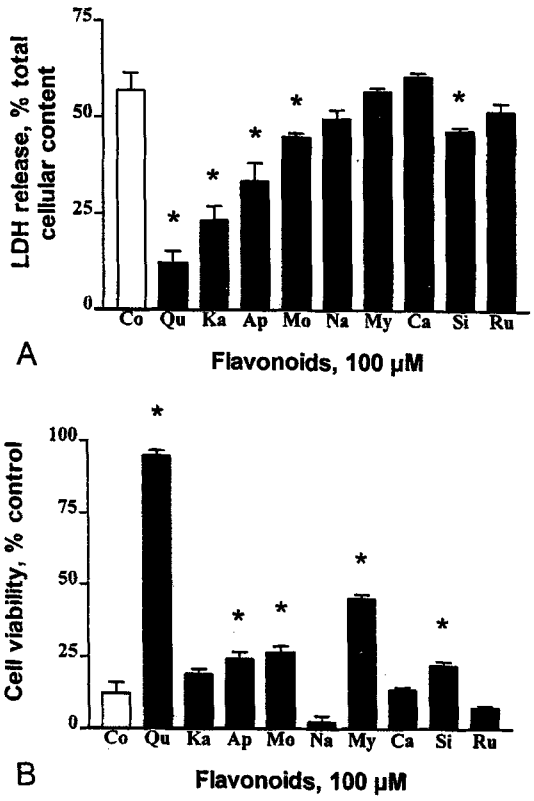
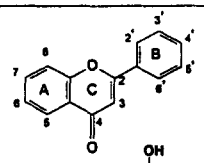
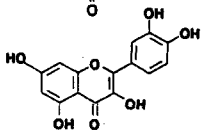
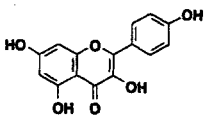
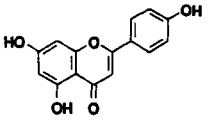
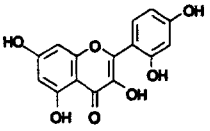
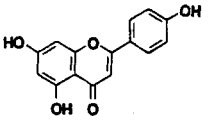
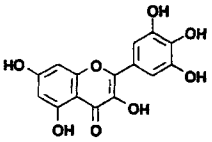
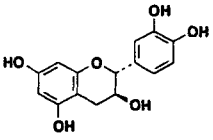
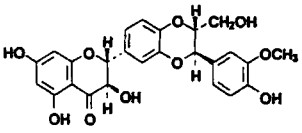
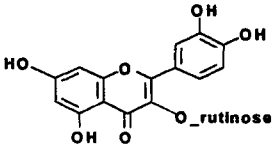


FIGURE 3 Protection from oxidative injury by various bioflavonoids: structure/activity analysis. Cell damage was assessed by LDH release (A) and MTT-test (B) 16 h following exposure of LLC-PK_1 cells to hypoxanthine (500 μM)/xanthine oxidase (50 mU/ml) for 60 min. Each condition represents mean ± SD of $n = 12$. Data were analyzed by ANOVA and the protective effect of each substance vs. vehicle-treated controls was analyzed by Dunnet's test. *$p < 0.05$ vs. control cells (abbreviations see Table I).

The results of the structure–activity studies lead to the hypothesis that the protective effects of quercetin not only depend on the number of hydroxyl substituents in the B- and C-ring, but also on the planarity and lipophilicity of the

TABLE I Structure and molecular weight of bioflavonoids under study

Substance	Structure	Molecular weight
Flavone, basic structure		
Quercetin (Qu)		302
Kaempferol (Ka)		286
Apigenin (Ap)		270
Morin (Mo)		302
Naringenin (Na)		272
Myricetin (My)		318
Catechin (Ca)		290
Silibinin (Si)		482
Rutin (Ru) (Quercetin-3-rutinoside)		611

substance, indicating that integration of quercetin into cellular membranes might be a prerequisite for its antioxidative capacity and that interruption of lipid peroxidation chain reactions might explain the protective potential of quercetin in our model.

Effects of Quercetin on Lipid Peroxidation

Lipid peroxidation was studied in three established models of oxidative injury (Figure 4). Hydrogen peroxide (H_2O_2, 1.5 mM) was either directly added to the medium or generated intracellularly by incubation with 100 μM 1-chloro-2,4-dinitrobenzene and 10 mM aminotriazole (CDNB + AT) which leads to glutathione depletion and catalase inhibition. Both models result in intracellular H_2O_2 accumulation and membrane lipid peroxidation. In contrast, treatment with the quinone menadione (2.0 mM) leads to intracellular generation of superoxide anion and H_2O_2, but cell injury occurs in the absence of lipid peroxidation due to direct protein arylation and protein crosslinking.[22–24]

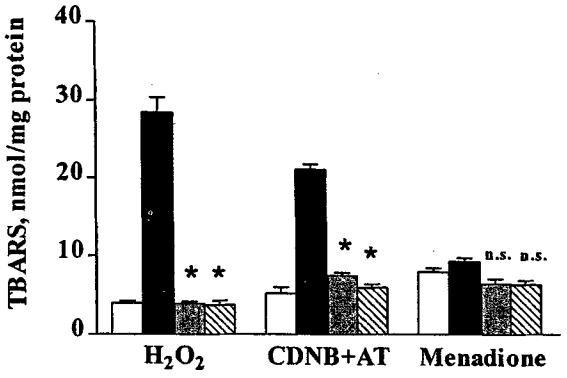


FIGURE 4 Effect of quercetin or DPPD on oxidant-induced lipid peroxidation, assessed by TBARS generation, in LLC-PK$_1$ cells. Monolayers were treated with either 1.5 mM H_2O_2, or 100 μM CDNB + 10 mM AT (CDNB + AT), or 2.0 mM menadione as described in the methods section. For each model TBARS accumulation in the supernatant of vehicle-pretreated (DMSO 0.2%; black bars), quercetin-pretreated (100 μM; shaded bars) and DPPD-pretreated (80 μM; hatched bars) cells is compared to non-damaged control cells (open bars). Each bar represents mean ± SD of $n = 12$. Statistical analysis was performed by ANOVA and the effects of quercetin or DPPD pretreatment vs. vehicle-pretreated cells were analyzed by Dunnet's test ($^*p < 0.05$).

The formation of TBARS, an indicator of lipid peroxidation, was significantly increased in cells treated with either 1.5 mM H_2O_2 for 3 h (4.0 ± 0.9 nmoles/mg protein (control) vs. 28.5 ± 6.9 (H_2O_2), $p < 0.05$) or CDNB + AT for 4 h (5.4 ± 2.6 (control) vs. 21.3 ± 2.1 (CDNB + AT), $p < 0.05$), whereas exposure to 2.0 mM menadione for 4 h did not alter cellular TBARS generation (8.0 ± 1.7 (control) vs. 9.6 ± 1.6 (menadione), p = n.s.). Pretreatment of cells with either 100 μM quercetin or 80 μM diphenyl-*p*-phenylenediamine (DPPD), an inhibitor of lipid peroxidation,[11] significantly lowered TBARS formation in the H_2O_2 as well as in the CDNB + AT model (3.9 ± 1.0 and 7.6 ± 1.1, resp.) but did not affect lipid peroxidation in the menadione model (6.7 ± 1.7 nmoles/mg protein).

The potential of quercetin to protect LLC-PK$_1$ cells from oxidative injury was examined in these models (Figure 5). In both models involving the induction of lipid peroxidation, i.e. H_2O_2 and CDNB + AT, structural and functional cell damage was significantly lowered by quercetin, whereas no protection was provided from menadione-induced cell injury which occurs in the absence of lipid peroxidation. Similarly, inhibition of lipid peroxidation by pretreatment of cell monolayers with DPPD, significantly lowered cell damage in the H_2O_2 and CDNB + AT models, but was without effect in the menadione model.

DISCUSSION

Within its class of naturally occurring flavan derivates, quercetin is a lipophilic, moderately soluble aglycon. Due to an optimal B/C-ring substitution pattern, quercetin is regarded as one of the most potent radical scavengers within the flavone series.[3,21] The B-ring (Table I) shows a 3′,4′-dihydroxy substitution pattern with three unsubstituted, electron-affinic carbon atoms. Its ring structure is conjugated and planar, thus

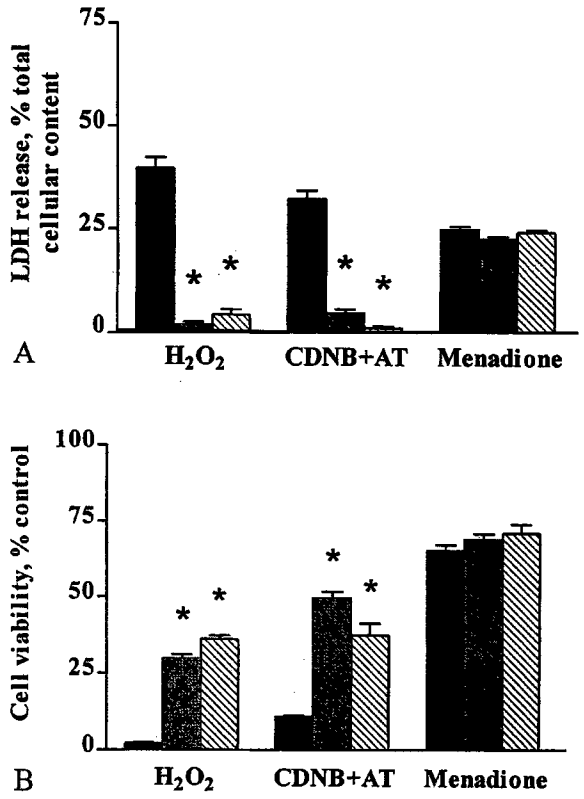


FIGURE 5 Effect of quercetin and DPPD on lipid peroxidation associated cell damage. Oxidative injury in LLC-PK_1 cells was induced by three different models as stated in Figure 4. Cell damage was assessed by LDH release (A) and MTT-test (B). In each model vehicle-pretreated cells (black bars) were compared to quercetin-pretreated (100 μM, shaded bars) or DPPD-pretreated (80 μM, hatched bars) cells. Each bar represents mean ± SD of $n = 12$. Statistical analysis was performed by ANOVA and the protective effects of quercetin or DPPD pretreatment vs. vehicle-pretreated cells were analyzed by Dunnet's test ($^*p < 0.05$).

allowing for maximum electron delocalization within the basic [flavone-]chromophore, a situation yielding a highly stable low-energy radical. Coplanarity of the B-ring with rings A and C depends on the substitution pattern of the B-ring and on substituents in 3-position of the C-ring.[21,25] Antioxidant activity of quercetin and other flavonoids is determined by several factors, of which lipophilicity (and thus uptake into the membranes, which are often the site of action), iron chelation, and scavenging of free radicals are the most important.[21]

Our comparative study strongly indicates that the protective activity of quercetin is related to the presence of two hydroxyl groups in 3′- and 4′-position of the B-ring and a hydroxyl group in 3-position of the C-ring in conjunction with a C2–C3 double bond of the basic flavone moiety. Omission of the B3′-hydroxyl group (kaempferol) or addition of a B5′-hydroxyl group (myricetin) significantly reduced protection from oxidative injury by lowering the number of electron-affinic B-ring carbon atoms and impairing radical scavenging properties. Overall electron affinity is further lowered by additional lacking of the C3-hydroxyl (apigenin) or by omitting the C-ring double bond (naringenin, catechin) and reduces antioxidative properties and protective activities. The finding that catechin was not protective stresses the importance of the oxo-extended C-ring chromophore for full protection.

Lipophilicity of flavones is negatively affected by the introduction of polar (rutin) or large, angular (silibinin) substituents. The finding that rutin completely lacked protective properties points to the importance of lipophilicity and suggests that membrane affinity may be essential for the protective effects of quercetin. Due to its steric geometry quercetin can easily interact with the ordered structure of lipid bilayers, whereas it has been demonstrated recently[26] that the non-planar quercetin glucoside rutin does not interact with biomembranes. The importance of the planar structure for antioxidative strength and possibly membrane incorporation is further stressed by the finding that morin, a compound with a similar number of hydroxyl groups as quercetin but lacking planarity, was not protective. Membrane affinity also seems to play an important role in cytoprotection provided by other polyphenols (caffeic acid, gallic acid and derivatives, ferulic acid, gossypol and ethyl protocatechuate).[27] Since all substances under study are excellent transition metal ion chelators, the differences in cytoprotection obviously cannot be accounted for by variations in chelating

activity. Otherwise all bioflavonoids with intact C-oxo-groups and/or vicinal dihydroxy substitution patterns should have shown protective activity similar to quercetin.

The hypothesis that quercetin exerts its protective activity via interference with lipid peroxidation was further studied in three models of oxidative injury differing in the importance of lipid peroxidation for cell damage. Exposure of cells to either H_2O_2 or CDNB + AT induces lipid peroxidation. In both cases it has been shown that lipid peroxidation is an early event preceding membrane damage and that inhibition of lipid peroxidation by specific inhibitors protects cells from further damage.[11] In contrast, intracellular generation of superoxide anion and hydrogen peroxide during metabolism of menadione results in lethal cell injury without induction of lipid peroxidation. Cell damage in this model is mainly due to protein arylation and protein crosslinking.[14]

Our data demonstrate the presence of lipid peroxidation in the H_2O_2 and the CDNB + AT model and the absence of lipid peroxidation in the menadione model. Pretreatment with quercetin resulted in significant reduction of TBARS generation in the two former models and had no influence on TBARS generation in the menadione model. It is important to point out that quercetin protected from structural and functional oxidative injury only in models where lipid peroxidation was induced, whereas it did not protect from lipid-peroxidation-independent cell injury. The effects of the lipid peroxidation inhibitor DPPD were similar to those of quercetin in each model. Also, DPPD did not ameliorate lipid-peroxidation-independent cell damage in the menadione model. Taken together, these data suggest that the protective activity of quercetin is dependent on the presence of lipid peroxidation and lead us to conclude that inhibition of lipid peroxidation may be the main mechanism of cytoprotection by quercetin.

Protective effects of various polyphenols against free-radical-mediated structural damage have been demonstrated *in vitro*[28] and in various cell types, s.a. Chinese hamster V79 cells[27] myocytes, endothelial cells, erythrocytes[29] or hepatocytes[30] and have been related to antioxidative and chelatory properties. In many of these studies[29,30] flavonoids were added at the time of oxidant injury. In these cases the mechanism of protection may be different from our system where cells were exclusively pretreated with bioflavonoids in order to avoid direct contact with oxygen radicals in the medium. The effects of bioflavonoids on the preservation of metabolic cell activity have not been studied so far. Our data demonstrate that cultured epithelial cells protected by quercetin also retain their metabolic activity. It has been shown *in vitro* that flavonoids are able to inhibit the peroxidation of phospholipid membranes[31] and lower microsomal and mitochondrial lipid peroxidation.[32,33] The specific mechanism by which integration of a highly active antioxidant into cellular membranes may protect from oxidative injury is not clear. Flavonoids may act as hydrogen-donating radical scavengers, for example, by scavenging lipid alkoxyl and peroxyl radicals, or by regenerating α-tocopherol through reduction of the α-tocopherol radical.[34] We favor the working hypothesis that quercetin quenches lipid peroxidation by forming a low-energy radical intermediate which is non-interferent with most functional structures of the fluid membrane and can eventually be eliminated by directed detoxification.

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