Free Radical Research, 2002 Vol. 36 (5), pp. 575–581

Taylor & Francis
healthsciences

Protection of Flavonoids Against Lipid Peroxidation: The Structure Activity Relationship Revisited

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Accepted by Professor B. Halliwell

(Received 25 July 2001; In revised form 26 October 2001)

The inhibition of the lipid peroxidation, induced by iron and ascorbate in rat liver microsomes, by phenols and flavones was studied. The activity of phenol was enhanced by electron donating substituents, denoted by the Hammett sigma (σ). The concentration of the substituted phenols giving 50% inhibition (IC_{50}) of lipid peroxidation gave a good correlation with the σ of the substituent ($\ln(1/IC_{50}) = -8.92\sigma + 5.80$ ($R = 0.94$, $p < 0.05$)). In flavones two pharmacophores for the protection against lipid peroxidation were pinpointed: (i) a catechol moiety as ring B and (ii) an OH-group at the 3 position with electron donating groups at the 5 and/or 7 position in the AC-ring. An example of a flavone with the latter pharmacophore is galangin (3,5,7-trihydroxyflavone) where the reactivity of the 3-OH-group is enhanced by the electron donating effect of the 5- and 7-OH-groups. This is comparable to the effect of electron donating substituents on the activity of phenol.

The prooxidant activity of flavones has been related to a low half peak oxidation potential ($Ep/2$). All flavones with a catechol as ring B have very low $Ep/2$, suggesting that they display a prominent prooxidant activity. In contrast, the $Ep/2$ varies within the group of flavones with a 3-OH, e.g. TUM 8436 (5,7,3′,4′-tetra-*O*-methyl-quercetin) has a relatively high $Ep/2$ and is an excellent protector against lipid peroxidation. Apparently amongst the flavones with the pharmacophore in the AC-ring there are good antioxidants that are expected to display no or limited prooxidant properties.

Keywords: Flavones; Lipid peroxidation; Structure activity relationship; Hammett σ; Anti-/prooxidant; Oxidation potential

INTRODUCTION

Flavonoids are a large heterogenic group of benzo-γ-pyron derivatives, which are abundantly present in our diet, e.g. in fruits and vegetables. Positive health effects of these compounds have been described in several diseases, such as pulmonary and cardiovascular diseases, and cancer.[1,2] In several food products flavonoids are the major antioxidants.[3,4] The antioxidant activity of flavonoids, reflected in, e.g. the protection of polyunsaturated fatty acids against free radical damage,[5–7] may explain their proclaimed health effects.[8,9]

Many structure activity relationships (SARs) have been proposed for the antioxidant activity of flavonoids.[10–13] In all these SARs the aromatic OH-groups play a pivotal role. It has even been suggested that the potency of a flavonoid correlates with the number of OH-groups.[11] Tentatively, this SAR is expected to be imprecise, e.g. it does not take the position of the OH-group in the molecule into account. Moreover, a SAR for an antioxidant can be complex since frequently several properties of a compound are involved. For example, in lipid peroxidation not purely the lipidperoxyl radical scavenging activity is studied. Also other properties such as lipophilicity, iron-chelation and prooxidant effects come into the equation. Thus the SAR for protection against lipid peroxidation is expected to be composed of the SARs of the different processes.

The aim of this study is to refine the SAR of a selected group of flavonoids, i.e. the flavonols, for the

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10715760290025951

inhibition of lipid peroxidation. Firstly, a series of simple phenols was tested to elucidate the influence of substituents. Subsequently, several flavones were evaluated.

MATERIAL AND METHODS

Chemicals

Phenol, catechol, hydroquinone, resorcinol, aminophenol, 2- and 4-methoxyphenol, 4-methylphenol, 3-hydroxyflavone, 5-hydroxyflavone, 7-hydroxyflavone, galangin and chrysin were purchased from Sigma (St. Louis, USA). TUM 8436 (5,7,3′,4′-tetra-*O*-methyl-quercetin) and TUM 8437 (penta-*O*-methyl-quercetin) were synthesized as previously described.[14] All other chemicals were of analytical grade.

Preparation of Microsomes

Microsomes were prepared from male Wistar rats, 200–250 g. After decapitation, the livers were removed and homogenized (1:2, w/v) in ice cold sodium phosphate buffer (50 mM, pH 7.4) containing 0.1 mM EDTA. The homogenate was centrifuged at 10,000*g* (20 min at 4°C). Subsequently the supernatant was centrifuged at 10,000*g* (20 min at 4°C) and again at 65,000*g* (60 min at 4°C). The microsomal pellet was resuspended in the phosphate buffer (2 g liver/ml) and stored at −80°C. Before use the microsomes were thawed and washed twice, with ice cold Tris–HCl buffer (50 mM, pH 7.4) containing 150 mM KCl.

Lipid Peroxidation

Microsomes (final concentration approximately 1 mg protein/ml) were incubated at 37°C, in a shaking water bath, with air being freely admitted in Tris–HCl/KCl (50 mM/150 mM, pH 7.4). Ascorbic acid (0.2 mM) was neutralized with KOH before addition. Reactions were started by adding a freshly prepared $FeSO_4$ solution (10 μM). Lipid peroxidation was assayed by measuring thiobarbituric acid (TBA)-reactive material.[15] The reaction in an aliquot of the incubation mixture (0.3 ml) was stopped by mixing with ice cold TBA–trichloroacetic acid (TCA)–HCl–butylhydroxytoluene (BHT) solution (2 ml). After heating (15 min, 80°C) and centrifugation (5 min) the absorbance at 535 versus 600 nm was determined. The TBA–TCA–HCl solution was prepared by dissolving 1.68 g TCA and 41.6 mg TBA in 10 ml 0.125 M HCl. BHT was dissolved in ethanol (1.5 mg/ml) and 1 ml of this solution was added to 10 ml TBA–TCA–HCl. The added chemicals did not interfere with the assay in the concentrations used. Results are expressed as mean ± SEM.

Half Peak Oxidation Potential

Flavonoids were dissolved in DMSO (final concentration 2.5% (v/v)), diluted 1:1 with nanopure water and further diluted in a 50 mM phosphate buffer, reaching a final concentration of 100 μM. Before starting the measurement, this buffer solution was purged with oxygen free nitrogen for 5 min after which the nitrogen was led over the solution. The measurements were performed with a platinum working electrode, a platinum counter electrode and a saturated calomel reference electrode (SCE). A scan was made from −0.2 to 0.6 V with a scanning speed of 20 mV/s on a PSTAT 10 potentiostat connected to an Autolab (Eco Chemie, Utrecht, The Netherlands) and controlled by the program General Purpose Electrochemical System 3.0 (Eco Chemie, Utrecht, The Netherlands).

When the flavonoid could not be oxidized between −0.2 and 0.6 V, a glassy carbon electrode was used as a working electrode in the range of −1 and 1 V. After each run the glassy carbon electrode was polished very thoroughly with 6 μ diamond spray on a polishing cloth and sonicated in ethanol, because of possible film formation and memory effects.

RESULTS

Phenols

Phenol itself is a poor inhibitor of lipid peroxidation. The concentration of phenol needed to inhibit 50% of the lipid peroxidation (IC_{50}) is 4050 μM. When the OH-group of phenol is methylated, the activity is extinguished (IC_{50} anisole > 7 mM). Hydroxylation of phenol (giving catechol, resorcinol or hydroquinone) leads to more active compounds (Fig. 1). OH-substitution at the 2 and 4 position of phenol

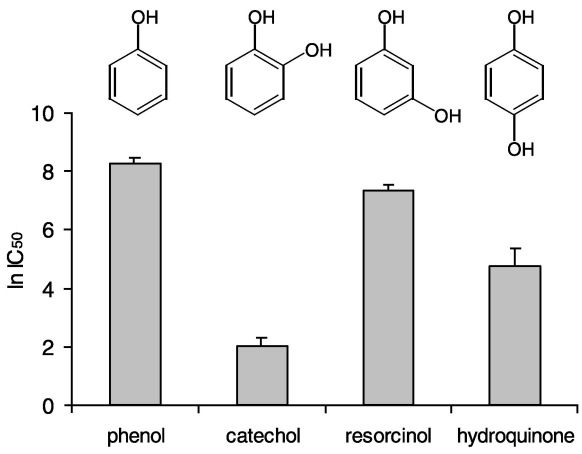

FIGURE 1 Protection against lipid peroxidation by several phenolic compounds. The activity is expressed as the natural logarithm of concentration of the compound that gives 50% inhibition of the lipid peroxidation (ln IC_{50}) mean ± SEM, $n = 3$.

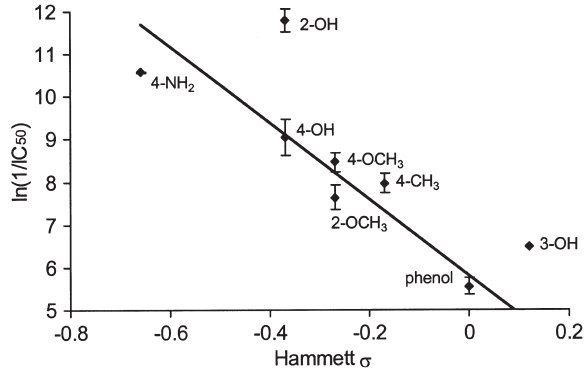

FIGURE 2 Correlation between Hammett σ and the protection of lipid peroxidation by substituted phenols ($\ln(1/IC_{50})$). The activity is expressed as the natural logarithm of 1/concentration of the compound that gives 50% inhibition of the lipid peroxidation ($\ln(1/IC_{50})$) mean ± SEM, $n = 3$. The equation of the fitted line is: $\ln(1/IC_{50}) = -8.92$ Hammett $\sigma + 5.80$ ($R = 0.94$, $p < 0.05$) Catechol, para-NH2-phenol and resorcinol were not included in the linear regression for the reasons described in the text.

increases the activity of phenol (IC_{50} catechol = 10 μM and hydroquinone = 156 μM). Hydroxylation at the 3 position of phenol also increases the activity, but this increase is much lower compared to substitution at position 2 or 4 (IC_{50} resorcinol = 1800 μM).

In the series of phenols containing various substituents a clear correlation between the IC_{50} and the Hammett σ is found (Fig. 2 and Eq. (1)).

$$\ln(1/IC_{50}) = -8.92 \text{ Hammett } \sigma + 5.80 \quad (R = 0.94,\ p < 0.05) \qquad (1)$$

Flavonols

Quercetin has a very high activity (Fig. 3). This activity is drastically decreased when all five OH-groups are methylated (TUM 8437, $IC_{50} > 2$ mM). Flavones with a catechol structure as ring B are also good protectors (rutin and monoHER, Fig. 3). But without a catechol structure as ring B (e.g. galangin) there also can be a high activity. In Fig. 4 several flavones not containing a catechol group are compared. Flavones containing the 3-OH group (galangin, kaempferol and triHEQ) were found to be better protectors compared to the corresponding compounds not containing this group (respectively, chrysin, apigenin and triHER).

Of the flavones containing only one OH-group in the AC-ring (Fig. 5) 3-OH-flavone ($IC_{50} = 22.8$ μM) was found to be the best protector. (IC_{50} 5-OH−flavone > 0.5 mM, 7-OH−flavone > 2 mM). TUM 8436, a compound possessing only a free OH-group at the 3 position, is also very active (IC_{50} = 13.5 μM).

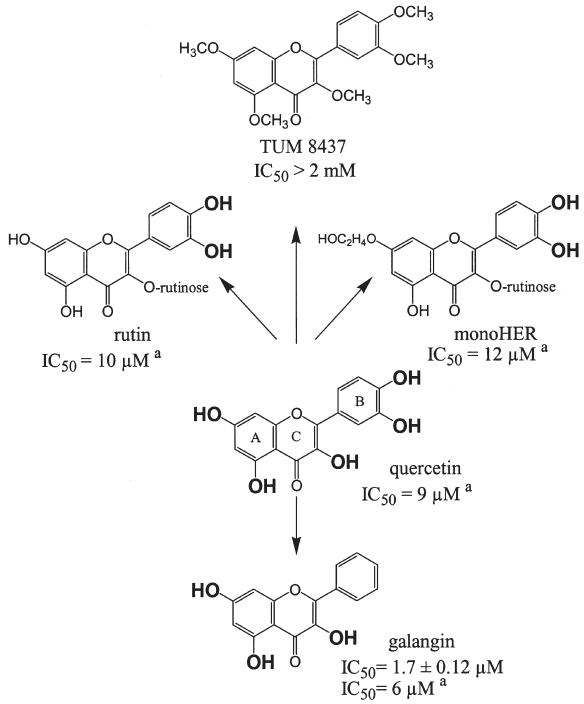

FIGURE 3 Protection against lipid peroxidation by several flavones. The activity is expressed as the concentration of the compound that gives 50% inhibition of the lipid peroxidation (IC_{50}). When all OH-groups of quercetin are methylated, the activity is drastically decreased. A flavone with a catechol group as ring B has high activity (rutin and monoHER). Galangin that only has OH-groups in the AC-ring also has a high activity in the inhibition of the lipid peroxidation (a) data taken from van Acker *et al.*[17]).

The activity of several flavones is correlated to the half peak oxidation potential ($Ep/2$) (Fig. 6). The group without the catechol and without the pharmacophore in ring AC has a relatively high $Ep/2$, the group of flavones with a catechol-group in ring B has a very low $Ep/2$. The group of flavones with only the pharmacophore in ring AC has a wide range of $Ep/2$.

DISCUSSION

This study on the SAR of the inhibition of lipid peroxidation by flavonols was started by characterizing the influence of substituents on the activity of phenol. It was found that the nature of the substituent as well as its position determines the activity of phenol. This influence can be explained by the different electron-donating effect of the various substituents at different positions. The Hammett σ denotes the electron donating ($-\sigma$) or withdrawing ($+\sigma$) effect of a certain substituent at a certain position. A clear correlation between the concentration needed to inhibit the lipid peroxidation for 50% (IC_{50}) and the σ is found in the series of substituted phenols (Fig. 2 and Eq. (1)).

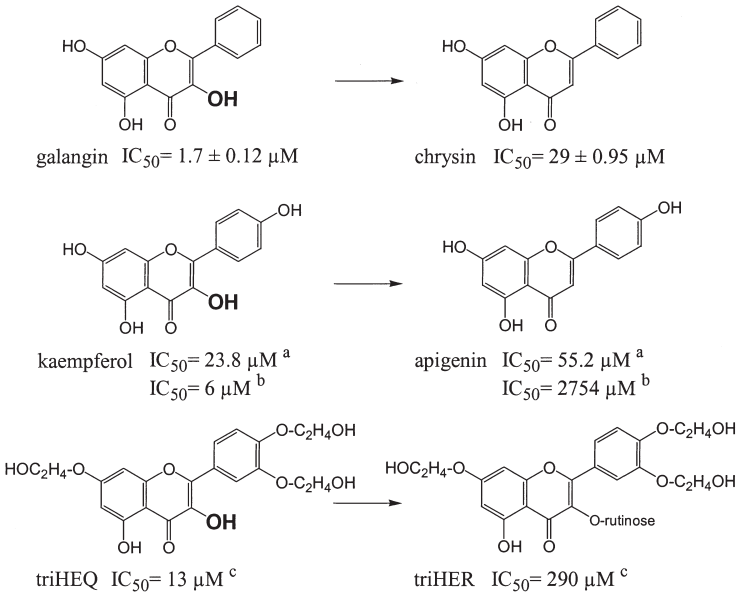

FIGURE 4 Protection against lipid peroxidation by several flavones. The activity is expressed as the concentration of the compound that gives 50% inhibition of the lipid peroxidation (IC_{50}). Galangin, kaempferol and triHEQ all have a high activity. When the 3-OH group is lacking, as in chrysin and apigenin or substituted as in triHER, the activity is diminished: (a) data taken from Mora *et al.*,[10] (b) data taken from van Acker *et al.*,[17] (c) data taken from Haenen *et al.*.[5]

The higher the electron-donating effect of a substituent, the higher is the protection of the substituted phenol against lipid peroxidation. Electron-donation weakens the O–H, bond making it easier to transfer an $H^{\cdot}$ to a lipidperoxyl radical, thus breaking the chain process of lipid peroxidation. The good correlation indicates that in this series of compounds the electron donating effect is the predominant factor for the protection against lipid peroxidation.

According to its σ, resorcinol is expected to be less active than phenol because of the electron with-

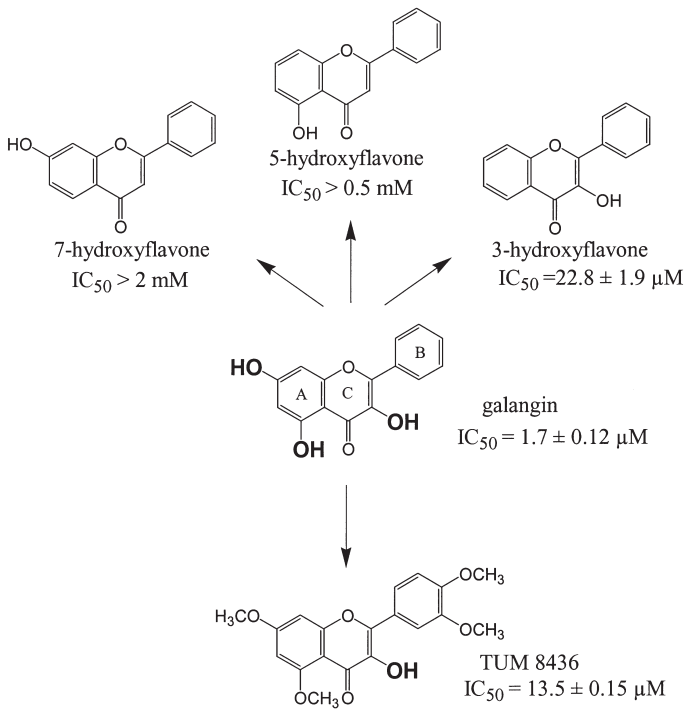

FIGURE 5 Protection against lipid peroxidation by several flavones. The activity is expressed as the concentration of the compound that gives 50% inhibition of the lipid peroxidation (IC_{50}). A compound with only one of the OH-groups of galangin has a poor activity. The rank order of activity is 3-OH-flavone > 5-OH-flavone > 7-OH-flavone. When 3-OH-flavone is substituted with electron donating groups, the activity is increased.

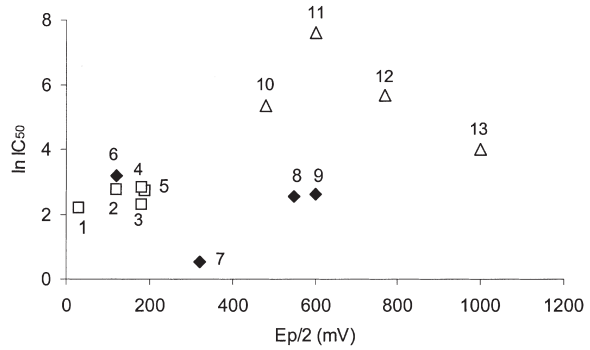

FIGURE 6 The correlation between the half peak oxidation potential ($Ep/2$) and the natural logarithm of the concentration that gives 50% inhibition of lipid peroxidation ($\ln(IC_{50})$). The flavonoids with a catechol group as ring B (□) are good inhibitors of lipid peroxidation and have a very low $Ep/2$. The flavonoids with the 3-OH group and electron donating substituents at position 5 and 7 (◆) have a high activity and a variable $Ep/2$. The flavonoids without both pharmacophores (Δ) are poor inhibitors and have a high $Ep/2$. The compounds are **1**: quercetin, **2**: fisetin, **3**: rutin, **4**: luteolin, **5**: monoHER, **6**: kaempferol, **7**: galangin, **8**: triHEQ, **9**: TUM 8436, **10**: diHER, **11**: TUM 8437, **12**: triHER and **13**: apigenin.

drawing effect of an OH-group at the 3-position. Surprisingly, resorcinol was found to be more protective than phenol (IC_{50} phenol = 4050 μM and resorcinol = 1800 μM). Apparently factors other than the electron-donating effect play a role in the inhibition of lipid peroxidation, e.g. lipophilicity, iron chelation, and location within the membrane.

Lipid peroxidation takes place in membranes and the scavenging of lipidperoxyl radicals can only be accomplished by the fraction of antioxidants that is present in the membrane. The lipophilicity of resorcinol ($\log P = 0.80$) is lower than that of phenol ($\log P = 1.46$), which means that a smaller fraction of resorcinol is present in the membrane compared to phenol. The relative high activity of resorcinol can therefore not be attributed to accumulation in the membrane. Multiple regression analyses did show no significant contribution of the lipophilicity on the activity in the series of substituted phenols. In the difference between the measured activity of 4-NH_2--phenol (IC_{50} = 25.6 μM) and the calculated activity based on the electron donating effect of a 4-NH_2--group (IC_{50} = 8.4 μM) lipophilicity is probably involved. 4-NH_2-phenol is a relatively hydrophilic compound with a $\log P$ of 0.04 versus $\log P$ 1.46 of phenol. This indicates that lipophilicity might play a role in lipid peroxidation.

Secondly, iron chelation can reduce radical generation in iron–ascorbate induced lipid peroxidation. This plays a pivotal role in the antioxidant effect of flavonoids.[5,16] Iron chelation has also been reported for catechol,[17] but has not been described for resorcinol. The higher protection against lipid peroxidation of catechol compared to its predicted activity based on the σ of an ortho OH-group (Fig. 2 and Eq. (1)) might be due to its iron chelating ability.

A third possibility for the relatively good activity of resorcinol might be found in the actual location of resorcinol within the membrane. For example, the chroman head of tocopherol, that is responsible for the actual antioxidant effect, is located in the membrane very near to the lipid–water interface.[18] Also for other antioxidants, such as carotenoids and 5-amino-salicylic acid, the position within the membrane is suggested to greatly influence their activity.[19,20] Resorcinol might have the structural elements that direct it to a position within the membrane where it can effectively protect against lipid peroxidation. Although this has not been studied, it might explain the higher activity of resorcinol.

In conclusion, in the series of the substituted phenols the electron-donating effect of the substituent appeared to be the most denominating factor in the inhibition of lipid peroxidation. Other factors including lipophilicity, iron chelation and location within the membrane probably are also involved in the final antioxidant effect, however, their exact role has not been elucidated.

Similar to phenol, the free OH-groups of flavonoids appear to be responsible for the activity. Polyphenols, such as quercetin are also reported to be good inhibitors of lipid peroxidation.[6,13,17] When all the OH-groups of quercetin are methylated, giving TUM 8437 (penta-*O*-methyl-quercetin), the activity is drastically decreased (Fig. 3, $IC_{50} > 2$ mM).

Flavonoids have been tested in many different antioxidant assays, e.g. TEAC and lipid peroxidation. Several SARs have been constructed. In these SARs the catechol moiety as ring B has been addressed as a pivotal group.[11,12,21–24] Indeed in all these studies, flavones with a catechol moiety in ring B were very good inhibitors of lipid peroxidation (IC_{50} is approximately 10 μM, Fig. 3).[10–12,17,21–23] We recently synthesized a series of 20 different flavones all containing a catechol group as ring B. Also these compounds appeared to be excellent protectors against lipid peroxidation, irrespective of the number and nature of the substituents at the AC-ring.[23] This denotes that the presence of a catechol group as ring B is indeed important. This is in line with the high activity of catechol we found in the series of substituted phenols.

Recently Pannala *et al.*[22] concluded that only the catechol moiety as ring B is important in the reactions of the $ABTS^{\cdot}$ with flavonoids. They found that also kaempferol, a compound having a phenol group as ring B, had an activity comparable to catechol containing compounds. The high potency of kaempferol was explained by the potential for conjugation between the 4′-OH-group in the B-ring and the 3-OH-group through the conjugated C-ring. In our study we found that besides kaempferol,

galangin (a flavonol lacking the 4′-OH-group of kaempferol) appeared also to be a potent protector against lipid peroxidation. Apparently the high activity of kaempferol and galangin does not reside in ring B but in another part of the molecule, i.e. the AC-ring.

To determine which OH-group in the AC-ring is the most important, 3-, 5- and 7-monohydroxyflavone were tested (Fig. 5). 3-Hydroxyflavone appears to be the most potent compound pointing to a pivotal role of the 3-OH-group. This is in line with the much higher potency of galangin, kaempferol and triHEQ ($IC_{50} = 1.7$, 23.8, and 13 μM, respectively, Fig. 4) compared to the corresponding compounds lacking a free 3-OH-group (i.e. chrysin, apigenin and triHER, $IC_{50} = 29$, 55.2, and 290 μM, respectively, Fig. 4)

Although 3-hydroxyflavone is a good protector against lipid peroxidation, its effectiveness is exceeded by galangin ($IC_{50} = 1.7\,\mu M$) more than 10 times. The explanation for the high activity of galangin can be found in the SAR of substituted phenols, described above. The activity of an aromatic OH-group is positively influenced by electron-donating substituents, e.g. the activity of phenol is drastically increased by substitution of an OH-group at the 2 or 4 position. The introduction of a 3-OH-group in phenol results only in a slight increase of the activity (Fig. 1). An even number of C-atoms between the electron donating group and the reactive OH-group appears to be optimal to increase the activity. An uneven number of C-atoms gives a suboptimal stimulation. This dependency on the number of C-atoms in-between is characteristic for an electron donating effect. In galangin there is an even number of C-atoms between the 3-OH-group and the 5- and 7-OH-groups. The interaction of the 5- and 7-OH-group on each other is suboptimal since they are separated by an uneven number of C-atoms. Moreover, the activity of the 3-monohydroxyflavone exceeds that of the 5- and 7-monohydroxyflavone. This indicates that the 3-OH-group in galangin is the most reactive one and that the OH-groups at the 5 and 7 position boost the reactivity of the 3-OH-group.

To examine this hypothesis the newly synthesized compound TUM 8436 (5,7,3′,4′-tetra-*O*-methyl-quercetin) was tested. This compound has only one free OH-group at the 3 position. At the 5,7, 3′, and 4′ position the flavone is substituted with an electron donating group, i.e. an OCH_3-group. The OCH_3-group was chosen because it has an electron donating effect comparable to the OH-group, although this effect is somewhat smaller (Hammett σ 4-$OCH_3 = 0.27$, 4-OH $= -0.37$). Moreover an OCH_3-group itself provides no protective effect against the lipid peroxidation, as shown by the high IC_{50} of TUM 8437 ($IC_{50} > 2$ mM, Fig. 3). TUM 8436 ($IC_{50} = 13.5\,\mu M$) proved to be a good inhibitor of lipid peroxidation, better than the 3-OH-flavone ($IC_{50} = 22.8$) (Fig. 5). This demonstrates that in the AC-ring the 3-OH is important for the antioxidant activity, and that the activity can be boosted by electron donating substituents (e.g. OH- or OCH_3-group) at the 5 and 7 position.

In the present study, it is shown that besides a catechol group as ring B another pharmacophore can be pinpointed, i.e. the 3-OH-group in the AC-ring in combination with electron donating groups. The SAR for the protection against lipid peroxidation is identical to the SAR for scavenging of peroxynitrite by flavones, where also these two pharmacophores have been identified.[14,25,26] In peroxynitrite scavenging it has also been shown that both pharmacophores can influence each other.[14] There are some striking differences in potency of some compounds in the protection against lipid peroxidation and peroxynitrite induced damage. Chrysin (5,7-hydroxyflavone) is practically inactive in peroxynitrite scavenging whereas it is a good protector against lipid peroxidation ($IC_{50} = 29.3\,\mu M$). Peroxynitrite scavenging, where only one activity is examined, is less complex than lipid peroxidation. As discussed above, the SAR for protection against lipid peroxidation is a combined SAR and several processes other than $H^{\cdot}$ donation come into the equation. Based on structural similarity, i.e. the structure in ring A resembles resorcinol, the same molecular mechanism responsible for the relative high potency of resorcinol might be involved in the unexpected high potency of chrysin.

Paradoxically, several flavones, e.g. quercetin, with an excellent antioxidant activity also display a prominent prooxidant activity. Several attempts were made by us using different types of assays, e.g. deoxyribose breakdown, coumarin hydroxylation, and capturing radicals with spintraps, to unravel radical formation and antioxidant effects. The net effect measured appeared to depend greatly on experimental conditions such as concentration of the compounds and the duration of the incubation. Apparently a straightforward quantification of the prooxidant activity of these compounds is hampered by interfering processes such as, the potent radical scavenging activity, iron chelation and the formation of active oxidation products from the flavones. Therefore it was decided to evaluate a crucial step prior to radical formation, i.e. the ability to act as a reductant to reduce transition metals like iron. This is an essential step in formation of radicals by, e.g. the Fenton reaction. The reducing ability of a compound is reflected by its $Ep/2$. Indeed a negative correlation between the prooxidant activity and the $Ep/2$ has been reported.[27,28]

In Fig. 6 the relation is shown between the concentration that gives 50% inhibition of the lipid

peroxidation (IC_{50}) and the $Ep/2$. This figure shows three groups. The flavones without the two pharmacophores are poor inhibitors of lipid peroxidation and have a high $Ep/2$. The flavones with a catechol moiety as ring B are very good inhibitors of lipid peroxidation and have a very low $Ep/2$, and thus are expected to be prooxidants. The flavones without the pharmacophore in ring B but with a 3-OH-group and electron donating groups at position 5 and 7 are also good inhibitors of lipid peroxidation but there is a wide variety in their $Ep/2$. Thus some of them are expected to have no or limited prooxidant properties. The independence of the activity and the $Ep/2$ of the latter group versus the low $Ep/2$ of all the flavones containing a catechol as ring B suggests that the two different pharmacophores act with a different mechanism to inhibit lipid peroxidation. This also means that it might be possible to unravel the pro- and antioxidant effects and to select good antioxidants which are expected to display no prooxidant effect, e.g. the semi-synthetic compound TUM 8436.

Acknowledgements

The authors like to thank Paul van Hal, Karlijn Gijzen and Julian Ramakers for their work on the prooxidant properties of flavonoids.

References

[1] Duarte, J., Perez-Vizcaino, F., Utrilla, P., Jiménez, J., Tamargo, J. and Zarzuelo, A. (1993) "Vasodilatory effects of flavonoids in rat aortic smooth muscle. Structure–activity relationships", *Gen. Pharmacol.* **24**, 857–862.

[2] Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B. and Kromhout, D. (1993) "Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study", *Lancet* **342**, 1007–1011.

[3] Rice Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M. and Pridham, J.B. (1995) "The relative antioxidant activities of plant-derived polyphenolic flavonoids", *Free Radic. Res.* **22**, 375–383.

[4] Paquay, J.B.G., Haenen, G.R.M.M., Korthouwer, R.E.M. and Bast, A. (1997) "Peroxynitrite scavenging by wines", *J. Agric. Food Chem.* **45**, 3357–3358.

[5] Haenen, G.R.M.M., Jansen, F.P. and Bast, A. (1993) "The antioxidant properties of five *O*-(β-hydroxyethyl)-rutinosides of the flavonoid mixture Venoruton", *Phlebology Suppl.* **1**, 10–17.

[6] Afanas'ev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A. and Potapovitch, A.I. (1989) "Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation", *Biochem. Pharmacol.* **38**, 1763–1769.

[7] Frankel, E.N., Kanner, J., German, J.B., Parks, E. and Kinsella, J.E. (1993) "Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine", *Lancet* **341**, 454–457.

[8] Massaeli, H., Sobrattee, S. and Pierce, G.N. (1999) "The importance of lipid solubility in antioxidants and free radical generating systems for determining lipoprotein peroxidation", *Free Radic. Biol. Med.* **26**, 1524–1530.

[9] Heller, F.R., Descamps, O. and Hondekijn, J.-C. (1998) "LDL oxidation: therapeutic perspectives", *Atherosclerosis* **137**(Suppl.), S25–S31.

[10] Mora, A., Paya, M., Rios, J.L. and Alcaraz, M.J. (1990) "Structure–activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation", *Biochem. Pharmacol.* **40**, 793–797.

[11] Cao, G., Sofic, E. and Prior, R.L. (1997) "Antioxidant and prooxidant behavior of flavonoids: structure–activity relationships", *Free Radic. Biol. Med.* **22**, 749–760.

[12] Arora, A., Nair, M.G. and Strasburg, G.M. (1998) "Structure–activity relationships for antioxidant activities of a series of flavonoids in a liposomal system", *Free Radic. Biol. Med.* **24**, 1355–1363.

[13] Ratty, A.K. and Das, N.P. (1988) "Effects of flavonoids on nonenzymatic lipid peroxidation: structure–activity relationship", *Biochem. Med. Metab. Biol.* **39**, 69–79.

[14] Heijnen, C.G.M., Haenen, G.R.M.M., Vekemans, J.A.J.M. and Bast, A. (2001) "Peroxynitrite scavenging of flavonoids: structure activity relationship", *Environ. Toxicol. Pharmacol.* **10**, 199–206.

[15] Haenen, G.R.M.M. and Bast, A. (1983) "Protection against lipid peroxidation by a microsomal glutathione-dependent labile factor", *FEBS Lett.* **159**, 24–28.

[16] Cheng, I.F. and Breen, K. (2000) "On the ability of four flavonoids, baicilein, luteolin, naringenin, and quercetin, to suppress the Fenton reaction of the iron–ATP complex", *Biometals* **13**, 77–83.

[17] van Acker, S.A.B.E., van den Berg, D.-J., Tromp, M.N.J.L., Griffioen, D.H., van Bennekom, W.P., van der Vijgh, W.J.F. and Bast, A. (1996) "Structural aspects of the antioxidant activity of flavonoids", *Free Radic. Biol. Med.* **20**, 331–342.

[18] Perly, B., Smith, I.C.P., Hughes, L., Burton, G.W. and Ingold, K.U. (1985) "Estimation of the location of natural alpha-tocopherol in lipid bilayers by ^{13}C-NMR spectroscopy", *Biochim. Biophys. Acta* **819**, 131–135.

[19] Pearson, D.C., Jourd'heuil, D. and Meddings, J.B. (1996) "The anti-oxidant properties of 5-aminosalicyclic acid", *Free Radic. Biol. Med.* **21**, 367–373.

[20] Woodall, A.A., Britton, G. and Jackson, M.J. (1997) "Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: relationship between carotenoid structure and protective ability", *Biochim. Biophys. Acta* **1336**, 575–586.

[21] Bors, W. and Michel, C. (1999) "Antioxidant capacity of flavanols and gallate esters: pulse radiolysis studies", *Free Radic. Biol. Med.* **27**, 1413–1426.

[22] Sekher Pannala, A., Chan, T.S., O'Brien, P.J. and Rice Evans, C.A. (2001) "Flavonoid B-ring chemistry and antioxidant activity: fast reaction kinetics", *Biochem. Biophys. Res. Commun.* **282**, 1161–1168.

[23] van Acker, F.A.A., Hageman, J.A., Haenen, G.R.M.M., van der Vijgh, W.J.F., Bast, A. and Menge, W.M.P.B. (2000) "Synthesis of novel 3,7-substituted-2-(3′,4′-dihydroxyphenyl)flavones with improved antioxidant activity", *J. Med. Chem.* **43**, 3752–3760.

[24] Bors, W., Heller, W., Michel, C. and Saran, M. (1990) "Flavonoids as antioxidants: determination of radical-scavenging efficiencies", *Meth. Enzymol.* **186**, 343–355.

[25] Heijnen, C.G.M., Haenen, G.R.M.M., van Acker, F.A.A., van der Vijgh, W.J.F. and Bast, A. (2001) "Flavonoids as peroxynitrite scavengers: the role of the hydroxyl groups", *Toxicol. In Vitro* **15**, 3–6.

[26] Haenen, G.R.M.M., Paquay, J.B.G., Korthouwer, R.E.M. and Bast, A. (1997) "Peroxynitrite scavenging by flavonoids", *Biochem. Biophys. Res. Commun.* **236**, 591–593.

[27] Dickancaite, E., Nemeikaite, A., Kalvelyte, A. and Cenas, N. (1998) "Prooxidant character of flavonoid cytotoxicity: structure–activity relationships", *Biochem. Mol. Biol. Int.* **45**, 923–930.

[28] Sergediene, E., Jönsson, K., Szymusiak, H., Tyrakowska, B., Rietjens, I.M.C.M. and Cenas, N. (1999) "Prooxidant toxicity of polyphenolic antioxidants to HL-60 cells: description of quantitative structure–activity relationships", *FEBS Lett.* **462**, 392–396.