Inhibition of Mammalian Thioredoxin Reductase by Some Flavonoids: Implications for Myricetin and Quercetin Anticancer Activity

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Abstract

The thioredoxin system, composed of thioredoxin reductase (TrxR), thioredoxin (Trx), and NADPH, exerts a wide range of activities in cellular redox control, antioxidant function, cell viability, and proliferation. Recently, the selenocysteine (Sec)-containing mammalian TrxR has emerged as a new target for anticancer drug development because TrxR and Trx are overexpressed in many aggressive tumors and the tumor cells seem to be more dependent on Trx system than normal cells. Here we have investigated the inhibition of mammalian TrxR by flavonoids which have been presumed to be cancer chemoprevention agents because of their antioxidant activities. Myricetin and quercetin were found to have strong inhibitory effects on mammalian TrxRs with IC50 values of 0.62 and 0.97 µM, respectively. The inhibition was shown to be concentration, NADPH, and time dependent and involved an attack on the reduced COOH-terminal -Cys-Sec-Gly active site of TrxR. Oxygen-derived superoxide anions enhanced the inhibitory effect whereas anaerobic conditions attenuated inhibition. Spectral analysis suggested that the flavonols might perform their inhibitory effects via semiquinone radicals. Additionally, the flavonols had the potential to inhibit the growth of A549 cells with the same potency as inhibition of TrxR. TrxR activity in the cell lysates was reduced on treatment with myricetin >50 µM, which coincided with the oxidation of Trx. The cell cycle was arrested in S phase by quercetin and an accumulation of cells in sub-G1 was observed in response to myricetin. Thus, the anticancer activity of quercetin and myricetin may be due to inhibition of TrxR, consequently inducing cell death. (Cancer Res 2006; 66(8): 4410-8)

Introduction

The thioredoxin system, composed of thioredoxin reductase (TrxR), thioredoxin (Trx), and NADPH, is critical for maintaining the cellular redox environment via its general protein disulfide reducing capacity. Thioredoxin can serve as an electron donor to ribonucleotide reductase, methionine sulfoxide reductase, and thioredoxin peroxidase (peroxiredoxin), and thus is involved in DNA synthesis, repair of methionine sulfoxide--oxidized proteins, or signaling via hydrogen peroxide (1–4). In addition, the thioredoxin system participates in many cellular signaling pathways by controlling the activity of transcription factors containing critical cysteines in their DNA-binding domains such as nuclear factor κB, activator protein-1, p53, and glucocorticoid receptor (5–8). Reduced Trx can bind and inactivate apoptosis signal-regulating kinase 1 whereas oxidation of Trx results in the activation of apoptosis signal-regulating kinase 1 and induction of apoptosis signal-regulating kinase 1-dependent apoptosis (9, 10).

Thioredoxin reductase is a ubiquitous flavoenzyme from archaea to human and the only enzyme able to catalyze the reduction of Trx by NADPH. Bacteria have a 70-kDa homodimeric TrxR with an active site disulfide and high substrate specificity. In contrast, mammalian TrxR is a larger 115-kDa homodimer with glutathione reductase–like structure (11, 12). Mammalian TrxR contains a conserved COOH-terminal active site sequence -Gly-Cys-Sec-Gly together with an NH2-terminal redox active disulfide (13–15). Besides Trx, the large TrxR has a broad spectrum of substrates, ranging from small molecules such as selenite, lipid hydroperoxides, ebelen, and dehydroascorbate to proteins like protein disulfide isomerase or glutathione peroxidase, etc. (16). Most of these substrates are involved in cellular redox regulation, therefore, TrxR plays a central role in maintaining the redox homeostasis directly or with Trx as well. TrxR and Trx have been reported to be overexpressed in many aggressive tumor cells in which the proliferation is crucially dependent on a constant deoxyribonucleotide supply (17–19). Accordingly, the inhibition of thioredoxin system can induce cell death or increase the tumor cell sensitivity to other anticancer drugs (18, 20–22).

More than 4,000 natural flavonoids are distributed in the plant kingdom and prokaryotes and have been consumed as foods or medical herbs by mankind since ancient time. Flavonoids have attracted a great deal of interest due to their extensive biological properties such as antioxidant, antiproliferative, anti-inflammatory, or antioxidant activity, which may contribute to their chemoprevention for the development of cancer and cardiovascular disease (23–25). However, the mechanisms of the exact effects of flavonoids are not fully understood and there are rare stringent pharmacologic and toxicologic tests for flavonoids thus far. Recently, several epidemiologic studies on the role of flavonoids have yielded contradictory results (26). Further careful investigations for the effects of flavonoids become significant because flavonoids have been well marketed as components of functional foods and are consumed from general diet.

The flavonoids, which are benzo-γ-pyrone derivatives with A, B, and C rings, are categorized as flavanones, flavones, flavonols, anthocyanidins, isoflavones, and flavonols (Table 1). They are the products of secondary metabolism from two main synthetic pathways by controlling the activity of transcription factors containing critical cysteines in their DNA-binding domains such as nuclear factor κB, activator protein-1, p53, and glucocorticoid receptor (5–8). Reduced Trx can bind and inactivate apoptosis signal-regulating kinase 1 whereas oxidation of Trx results in the activation of apoptosis signal-regulating kinase 1 and induction of apoptosis signal-regulating kinase 1-dependent apoptosis (9, 10).

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Inhibition of Mammalian TrxR By Flavonoids

**Materials and Methods**

**Chemicals and enzymes.** The preparation of recombinant rat TrxR and Sec<sup>463</sup>—Cys mutant TrxR was previously described (30, 31). The recombinant rat TrxR yielded a specific activity of 50% of wild-type TrxR as determined by 5,5'-dithiobis-(2-nitrobenzoic acid) assay. Concentrations of the enzymes were measured using ε<sub>413</sub> = 11.3 (mmol/L)<sup>−1</sup> cm<sup>−1</sup>, the absorbance of FAD. Quercetin, kaempferol, taxifolin, rutin, apigenin, luteolin, daidzein, genistein, catechins, pelargonidin, NADPH, insulin, and 5,5'-dithiobis-(2-nitrobenzoic acid) were obtained from Sigma. Myricetin was from Fluka. 4-Acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid (AMS) and biotin-conjugated iodoacetamide were purchased from Molecular Probes. The flavonoids were dissolved in fresh DMSO or methanol and concentrations of DMSO or methanol were <5% solvent buffer. All the other reagents were of analytic grade.

pathways: the shikimate and the acetate pathways (27). One of the main actions of flavonoids is antioxidant activity; in some cases, they exhibit pro-oxidant properties (28, 29). Here we have studied the interactions of all six sorts of flavonoids with the thioredoxin system to elucidate the molecular mechanism of these promising drug candidates. We found that 3-hydroxyl-containing flavonoids such as quercetin, myricetin, taxifolin, catechin, and pelargonidin exhibited an NADPH-, concentration-, and time-dependent inhibitory effect. The inhibitory capacity on TrxR correlated with the main actions of flavonoids is antioxidant activity; in some cases, they exhibit pro-oxidant properties. We found that 3-hydroxyl-containing flavonoids such as quercetin, myricetin, taxifolin, catechin, and pelargonidin exhibited an NADPH-, concentration-, and time-dependent inhibitory effect.

**Table 1. Interactions of mammalian TrxR with flavonoids**

<table>
<thead>
<tr>
<th>Classes</th>
<th>Compounds</th>
<th>Substitutions</th>
<th>TrxR IC&lt;sub&gt;50&lt;/sub&gt; (µmol/L)</th>
<th>NADPH oxidase activity (ΔA&lt;sub&gt;340&lt;/sub&gt;/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonols</td>
<td>Myricetin</td>
<td>3,5,7,3,4,5'-OH</td>
<td>0.62</td>
<td>0.0120 (0.0125)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>3,5,7,3,4'-OH</td>
<td>0.97</td>
<td>0.0024 (0.0045)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Kaempferol</td>
<td>3,5,7,4'-OH</td>
<td>1.000</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Rutin</td>
<td>3-rutinose, 5,7,3,4'-OH</td>
<td>1.000</td>
<td>ND</td>
</tr>
<tr>
<td>Flavanones</td>
<td>Taxifolin</td>
<td>3,5,7,3,4'-OH</td>
<td>6.6</td>
<td>ND</td>
</tr>
<tr>
<td>Flavanols</td>
<td>Catechin</td>
<td>3,5,7,3,4'-OH</td>
<td>4.2</td>
<td>ND</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td>Pelargonidin</td>
<td>3,5,7,4'-OH</td>
<td>6.2</td>
<td>ND</td>
</tr>
<tr>
<td>Flavones</td>
<td>Luteolin</td>
<td>5,7,3,4'-OH</td>
<td>250</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Apigenin</td>
<td>5,7,4'-OH</td>
<td>500</td>
<td>ND</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Genistein</td>
<td>5,7,4'-OH</td>
<td>2000</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Daidzein</td>
<td>7,4'-OH</td>
<td>2000</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Value represents the presence of 1 µmol/L E. coli Trx in the reaction solution.

<sup>a</sup> ND, no activity was detected.

**5,5'-Dithiobis-(2-nitrobenzoic acid) reduction assay.** NADPH-reduced recombinant TrxR was preincubated for 1 to 2 hours with flavonoids at room temperature; then TrxR activity was assayed by 5,5'-dithiobis-(2-nitrobenzoic acid) assay in the solution containing 50 mmol/L Tris-HCl (pH 7.5), 200 µmol/L NADPH, 5 mmol/L 5,5'-dithiobis-(2-nitrobenzoic acid), and 1 mmol/L EDTA (30). The absorbance at 412 nm was followed with an Ultraspex 3000 UV/Visible spectrophotometer (Pharmacia Biotech) or a VERSA microplate reader (Molecular Devices). TrxR activity was obtained by measuring the slope of absorbance change in the initial two minutes.

**NADPH oxidase activity assay.** NADPH oxidization was done in Tris-HCl buffer (pH 7.5) containing 200 µmol/L NADPH and 1 mmol/L EDTA by following the absorbance at 340 nm in the presence of 50 µmol/L flavonoids and 220 nmol/L rTrxR for 1 hour. The same solutions without TrxR or without flavonoids were used as the controls. NADPH oxidase activity was defined as the value of ΔA<sub>340</sub>/min of the initial 20 minutes and obtained by subtracting the values of the controls.

**Incubation under anaerobic condition.** TrxR (330 nmol/L) was reduced by NADPH at room temperature for 30 minutes. Then the solution was saturated with nitrogen for 15 minutes and incubated with 50 µmol/L flavonoids. Aliquot of solution was withdrawn at different time points to measure TrxR activity.

**Effect of reactive oxygen species generated by xanthine/xanthine oxidase system on the inhibition of TrxR by myricetin.** NADPH-reduced TrxR (330 nmol/L) was incubated with 50 µmol/L flavonoids, alone or in the presence of 100 µmol/L xanthine, 0.04 units/mL xanthine oxidase, or xanthine (100 µmol/L)/xanthine oxidase (0.04 units/mL) for 15 minutes. Then, 20 µL of the solutions were picked up for TrxR activity assay.

**Absorption spectra.** UV-Vis spectra were recorded with a Shimadzu UV-2100 spectrophotometer with a 1-cm quartz cuvette at room temperature.
Myricetin (100 μmol/L) was incubated in 50 mmol/L Tris-HCl (pH 7.5) containing 200 μmol/L NADPH and 1 mmol/L EDTA by following the decrease of absorbance at 340 nm for the inhibition of DTT-reduced TrxR (data not shown), suggesting that NADPH is another factor involved in the inhibition reaction directly.

Effect of reactive oxygen species on the inhibition of TrxR by flavonoids. Accumulating evidence show that reactive oxygen species (ROS) may be produced when the mammalian TrxR is chemically modified (21, 33–35). We checked the effect of ROS on the interaction of TrxR with flavonoids. Anaerobic incubation reduced the inhibitory effect for the effective flavonoids (Fig. 2B). Moreover, the presence of superoxide dismutase diminished the inhibition dramatically, maintaining the color of the reaction solutions as yellow (Fig. 2B). In the absence of superoxide dismutase, the reaction mixture changed to dark, suggesting that flavonoids were oxidized during the inhibition process and superoxide anions were involved in the inhibitory process. In contrast, catalase did not show a significant effect to prevent the inhibition as superoxide dismutase. Furthermore, ROS generated by xanthine/xanthine oxidase enhanced the inhibitory effect of flavonoids (Fig. 2C).

Interactions between flavonoids with TrxR. The results described above obviously show that ROS played a critical role in the inhibition of TrxR by flavonoids. Flavonols can be oxidized to give rise to semiquinone, quinone, or quinone methide with a new peak around 490 nm (Fig. 3A; refs. 36–38). Interestingly, the presence of active TrxR prevented the oxidation of myricetin, which might be due to a reductive activity of TrxR on oxidized flavonoid products. A new absorbance peak appeared only when TrxR was inhibited to <10% of the original TrxR activity (Fig. 3B). NADPH oxidation activity of TrxR was also assayed in the presence of flavonoids by following the decrease of absorbance at 340 nm.
Myricetin was the best substrate of TrxR among the flavonoids. No increased NADPH oxidase activity was observed for taxifolin, rutin, apigenin, luteolin, daidzein, genistein, catechin, pelargonidin, and kaempferol (Table 1). Flavonols myricetin and quercetin may have some ROS-reducing activity via semiquinone as redox cycling molecules. Then the properties that the two flavonols are the substrate of Trx system will contribute to their effects as excellent antioxidants.

(Fig. 3D). Myricetin was the best substrate of TrxR among the flavonoids. No increased NADPH oxidase activity was observed for taxifolin, rutin, apigenin, luteolin, daidzein, genistein, catechin, pelargonidin, and kaempferol (Table 1). Flavonols myricetin and quercetin may have some ROS-reducing activity via semiquinone as redox cycling molecules. Then the properties that the two flavonols are the substrate of Trx system will contribute to their effects as excellent antioxidants.

The attacking site of mammalian TrxR by the flavonoids. A major structural difference between mammalian and bacterial TrxR is the penultimate COOH-terminal selenocysteine in mammalian TrxR, which is essential for its Trx reduction activity (13, 39). When TrxR is in its reduced form, without a protective selenenylsulfide bridge with the neighboring Cys residue, the selenocysteine is deprotoned and becomes a highly active nucleophilic agent because of its low theoretical pKa value of 5.3. The Sec498→Cys mutant TrxR enzyme cannot prevent the oxidization of myricetin, indicating that Sec498 plays a key role in the interaction between TrxR and flavonols (Fig. 3C). To further clarify whether the active site is involved in the inhibition, we determined the free selenocysteine by blotting with biotin-conjugated iodoacetamide. As shown in Fig. 3D, the bands representing selenol of selenocysteine in both dimeric 115-kDa and monomeric 57-kDa TrxRs decreased with increasing incubation time, consistent with the loss of activity. Both labeled monomer and dimer TrxR bands disappeared after the incubation with myricetin for 2 hours, but were present in the control samples with DMSO treatment. Other flavonoid inhibitors such as quercetin showed the same property as myricetin. In contrast, flavonoids without inhibitory effect showed the same negative result as with DMSO. Furthermore, the flavonols myricetin and quercetin did not exhibit strong inhibition on E. coli TrxR, which has the FAD and NADPH binding domains but lacks the selenocysteine active site (data not shown). These results strongly suggest that the flavonoids attack the selenocysteine in the active site, causing irreversible inhibition of TrxR.
Effects of flavonols on the cells \textit{in vitro}. TrxR is overexpressed in A549 cells (40) so that this cell line is a good model to study TrxR-regulating cellular activity. We investigated the effect of different flavonoids on A549 cell growth using XTT method to measure cell viability. Myricetin exhibited the strongest toxic effect on A549 cells, consistent with the inhibition of TrxR \textit{in vitro} by myricetin, which displayed the lowest IC$_{50}$ and was the fastest inhibitor among the flavonoids. Quercetin showed some toxicity to A549 cells at higher concentrations. Mouse fibroblast cells were shown to be more resistant to the two flavonols (Fig. 4A). We also measured the TrxR activity and determined the redox state of thioredoxin. At low concentration of myricetin and quercetin (0-12 μmol/L), TrxR activity of cell lysate did not show significant changes after 24 hours of incubation. When A549 cells were treated with higher concentrations of myricetin and quercetin, cell death was induced and TrxR activity in cell lysates decreased with increasing flavonoid concentration. Trx redox state was detected by electrophoresis migration shift in nonreducing gel. The alkylation agent AMS was used to trap the free thiols of Trx under denaturing conditions (41) in which oxidized Trx migrated faster than the reduced form. In the control A549 cells treated with DMSO or the cells treated with 25 μmol/L myricetin or quercetin, only reduced Trx was detected. Treatment with 50 or 75 μmol/L myricetin resulted in the reduction of reduced protein level and appearance of oxidized Trx (Fig. 4B and C). Myricetin and quercetin had the same ability to inhibit TrxR \textit{in vivo}; however, myricetin possessed much stronger cytotoxicity than quercetin. The difference in cytotoxicity may be due to the capacity of myricetin to produce more ROS (42) and the presence of high level of TrxR in the cells, resulting in the ROS-mediated cell death. To clarify whether the myricetin/quercetin–induced cell death resulted from the reduced DNA synthesis and/or a redox state change, we analyzed the cell cycle status of the A549 cells exposed to flavonoids. As shown in Fig. 4D, compared with the control A549 cells treated with DMSO, A549 cells displayed an S-phase arrest after the treatment with 50, 75, and 100 μmol/L of quercetin for 48 hours; correspondingly, an accumulation of DNA related to sub-G$_1$ fraction was seen after exposure for 48 hours to 25, 50, and 75 μmol/L myricetin. This result suggests that cell death induced by myricetin may result from redox changes via the inhibition of Trx system, whereas cell death induced by quercetin is due to inhibition of Trx and subsequent blockage of DNA synthesis via inhibition of ribonucleotide reductase.

Discussion

It is well known that dietary foods rich in flavonoids have beneficial effects on human health, including the reduction of
the incidence of cancer and cardiovascular diseases (43). Our present study shows that flavonoids, especially myricetin and quercetin, can irreversibly inhibit mammalian TrxR under physiologic conditions in a time-, NADPH-, oxygen-, and concentration-dependent manner. ROS participate in the inhibition process and the mechanism of flavonols may occur as a result of their easy oxidization to flavonol semiquinone species. Why myricetin and quercetin differ from the other compounds may be because these two flavonols are readily auto-oxidizable forming superoxide radicals. Some quinones have been previously shown to be either the substrates or inhibitors of TrxR (44). Our results show that the flavonols myricetin and quercetin and their oxidized products are both inhibitors and substrates. Taken together, we propose several possible interactions between flavonols quercetin and myricetin with TrxR (Scheme 1). The interactions of flavonols with TrxR may occur in several steps. Step 1, flavonols inhibit TrxR directly and produces modified TrxR, triggering the inactivation of TrxR. Step 2, modified TrxR produces oxygen radicals or ROS. Step 3, the oxygen radicals attack the flavonols to yield α-semiquinone or via auto-oxidization. Step 4, α-semiquinone reacts with active TrxR and inhibits it. Step 5, α-semiquinone can be oxidized further to be quinone methide, an electrophile which can form a conjugate with protein thiols (45). Step 6, the oxidation of step 5 can be prevented by the active TrxR, which can be inactivated by quinone methide in reverse. Superoxide dismutase or incubation under anaerobic conditions will attenuate superoxide production and diminish the step 4 reaction, whereas xanthine/xanthine oxidase system produces more superoxide and accelerates the reaction of this step. The semiquinone or quinone methide may attack the selenocysteine in

Figure 4. Effects of myricetin and quercetin on the TrxR activity and redox state of Trx in cells in vitro. A, A549 cells were incubated in serum-free medium with different concentrations of flavonoids for 24 hours. Then the cell viability was detected by XTT method. After XTT agents were added, the cells were grown for another 4 hours. Mouse embryo fibroblast cells were used as the control normal cells. Points, mean of four experiments; bars, SD. B, A549 cell were treated with different concentrations of flavonoids in serum-free medium for 24 hours and lysed in lysis buffer. The cells lysates were subjected to TrxR activity assay. Columns, mean of three experiments; bars, SD. C, flavonoid-treated A549 cells were lysed in guanidine lysis buffer containing 15 mmol/L AMS. The proteins were separated with a 12% Bis-Tris gel with MES running buffer and detected by anti-human Trx antibody. D, analysis of cell cycle of A549 cells treated with myricetin and quercetin.
COOH terminus of reduced TrxR to modify TrxR and prevent the enzyme from reduction of Trx. Consequently, reduced Trx, which is normally present in the cells (46) as a result of TrxR activity, will be replaced by the oxidized form, which may induce Trx-mediated cell death.

Thioredoxin system and reduced glutathione-glutaredoxin system (NADPH, glutathione reductase, glutathione, and glutaredoxins) are the two major pathways to provide electrons for ribonucleotide reductase, which is essential for DNA synthesis (2). Here we have shown inhibition of TrxR by flavonols. Considering that flavonols also have the reported activity to inhibit glutathione reductase (47), one toxic effect of flavonols on the cells may be via the inhibition of DNA synthesis. We have also shown here that quercetin can arrest A549 cells in S phase, which is consistent with previous reports (48, 49). The DNA replication rates of gastric cancer cells were found to be reduced to 14% of the control by 70 μmol/L quercetin, which may be responsible for the delay of cell division (49). This can be explained by TrxR inhibition by quercetin and thus a change of reduced Trx into its oxidized form. The redox state change will make the cell unable to supply enough electrons to ribonucleotide reductase for a constant DNA synthesis in tumor cells because Trx is a major high-capacity electron donor for mammalian ribonucleotide reductase (1, 2, 4).

Another cytotoxic activity of flavonols may be due to the production of ROS which can cause the cell apoptosis (50). Our results show that inhibition of TrxR by flavonols is dependent on the redox environment. TrxR inhibition was remarkably reduced under nitrogen saturation and superoxide dismutase–containing solution; in contrast, superoxide enhanced the inhibition dramatically. It has been reported that inhibition of TrxR by dinitrohalobenzenes leads to induction of NADPH oxidase, producing ROS (35). When TrxR is inhibited by semiquinone or quinone methide from flavonol oxidation by ROS, the ROS production and TrxR inhibition would be amplified by redox cycling (Scheme 1). In malignant tumor cells, the antioxidant systems are elevated perhaps to balance the high level of oxidant species (51–53). The elevation may deplete antioxidative capacity in tumor cells, and more ROS production will thus lead the cells to redox signaling–mediated apoptosis (50). The observation that myricetin induced the cells in sub-G1 fraction may be one example of ROS-mediated cell death because myricetin more readily formed oxygen radicals.

Several epidemiologic studies have been done to investigate the roles of flavonoids in the human disease prevention. Although no definite conclusion can be made thus far, these results suggest that lung cancer risk is reduced by the intake of flavonols (43, 54). Compared with other tissues, lung is exposed to oxygen directly. Thus, lung cells get a stringent oxidative stress and antioxidant enzymes are expressed to make a redox balance. Because most of natural flavonoids are found to be conjugated with glycoside (23), the antioxidant and pro-oxidant activities of flavonoids in vivo have been challenged. Our data also show that rutin, the glycosylated form of quercetin, does not show any inhibitory effect on TrxR. However, recently, a study of the tissue distribution of quercetin shows that the highest levels of quercetin metabolites are located in the lungs of rats. The report also shows that the lung has a high deconjugation activity. The aglycone concentration in the lung extracts ranged from 15% to 22% whereas only 0.4% to 0.8% was found in plasma (55). Therefore, the fact that TrxRs are overexpressed in lung cancer cells (40, 56) should be a reasonable explanation for lung cancer chemoprevention by the flavonols myricetin and quercetin because flavonols most probably exert their inhibitory effects via semiquinone or quinone methide and the high oxygen concentration in lung can easily oxidize flavonols into semiquinone/quinone methide. Flavonols at low concentration are the substrates of TrxR and...
enhance the lung antioxidant activity, whereas high concentrations of flavonols will be changed to become pro-oxidant and induce the malignant cell death via the high expression level of TrxR, particularly in the cells of epithelial origin (57, 58). This property of myricetin is probably also the reason that higher myricetin intake may be correlated with lower prostate cancer risk (43). In summary, our results clearly show that the toxicity of flavonols is due to their pro-oxidant activity and some flavonoids were shown to inhibit mammalian TrxRs; in particular, myricetin and quercetin were the strongest inhibitory flavonols among the flavonoids. Superoxide anions were involved in the inhibited process and the inhibition of TrxR and ROS production by the modified enzyme (21) induced the oxidation Trx in the cells. These results correlated the anticancer activity of flavonoids with the large number of heteroaromatic quinols and may yield a new strategy for the drug design in cancer therapy.

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References


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