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Inhibitory Effects of Flavonoids on Rabbit Heart Carbonyl Reductase

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The inhibitory effects of flavonoids (galangin, kaempferol, quercetin, myricetin, morin, and taxifolin) on rabbit heart carbonyl reductase (RHCR) were investigated using 4-benzoylpyridine (4BP) as the substrate. The stereochemical characteristics of the flavonoids were found to be a factor determining their inhibitory potencies toward RHCR. Furthermore, the lipophilicity, and the scavenging or antioxidative effects of the flavonoids were likely to complicate the structure-activity relationship of their inhibitory effects on RHCR. Quercetin inhibited RHCR uncompetitively with respect to NADPH and competitively with respect to 4BP, suggesting that it competes with 4BP at the substrate-binding site of RHCR. RHCR efficiently reduced benzoquinones (1,4-benzoquinone and 2-methyl-1,4-benzoquinone) and naphthoquinones (1,4-naphthoquinone and menadione). Galangin was a potent inhibitor of RHCR when menadione was used as the substrate, and prevented the formation of the superoxide anion radical in the presence of RHCR, NADPH, and menadione. Flavonoids may be useful compounds for suppressing the cardiotoxicity of quinones by inhibiting RHCR.

Key words: flavonoid, inhibition mechanism, rabbit heart carbonyl reductase, structure-activity relationship, superoxide anion radical.

Carbonyl reductase [EC 1.1.1.184] is an enzyme responsible for the NADPH-dependent reduction of endogenous and exogenous carbonyl compounds to the corresponding alcohol metabolites (*1–3*). So far, a variety of carbonyl reductases have been purified from the liver, kidney, brain, lung, and ovary of mammalian species (*4–10*). We recently purified a new carbonyl reductase to homogeneity from the cytosolic fraction of rabbit heart, using 4-benzoylpyridine (4BP) as the substrate (*11*). The rabbit heart carbonyl reductase (RHCR) efficiently reduced not only ketones and aldehydes, but also menadione (2-methyl-1,4-naphthoquinone, vitamin K_3). It is of interest that RHCR functions as a quinone reductase (*12*).

Quinones including menadione have been reported to exert toxic effects in biological systems (*13–15*). The toxicity of quinones is known to be induced through several different mechanisms, an important mechanism being the formation of the superoxide anion radical during the redox (oxidation-reduction) cycling of quinones. Quinones are enzymatically reduced to semiquinones and hydroquinones, respectively, by a one-electron transfer reductase, *e.g.*, NADPH-cytochrome P450 reductase [EC 1.6.1.4], and by a two-electron transfer reductase, *e.g.*, NAD(P)H quinone oxidoreductase (DT-diaphorase) [EC 1.6.99.2]. Semiquinones readily react with molecular oxygen by forming the superoxide anion radical and by regenerating the parent quinones, whereas hydroquinones are relatively stable as to autoxidation. Interestingly, Jarabak and Harvey (*16*, *17*) have demonstrated that although human placental carbonyl reductase catalyzes the two-electron reduction of quinones, it mediates the redox cycling of quinones and leads to the formation of the superoxide anion radical. We also found that RHCR produces the superoxide anion radical in the presence of menadione and NADPH (*11*).

Flavonoids are a group of compounds ubiquitously found in vegetables, fruits, and beverages such as tea and red wine. It has been reported that flavonoids inhibit a variety of enzymes (*18–22*). Among flavonoids, only quercetin and quercitrin (quercetin-3-L-rhamnoside) are well-known inhibitors of carbonyl reductases purified from several tissues (*4–8*, *10*, *11*), but information on the inhibition of carbonyl reductase by flavonoids other than quercetin and quercitrin has been very limited. Flavonoids may inhibit RHCR and prevent the formation of the superoxide anion radical during the redox cycling of quinones catalyzed by the enzyme. The purpose of the present study was to elucidate the detailed mechanism and pharmacological significance of the inhibition of RHCR by flavonoids.

MATERIALS AND METHODS

Materials—4BP was purchased from Wako Pure Chemicals (Osaka). Flavonoids were obtained from the following sources: galangin and myricetin from Aldrich (Milwaukee, WI, USA); morin and taxifolin (racemate) from Sigma (St. Louis, MO, USA); kaempferol from Tokyo Kasei (Tokyo); and quercetin from Wako Pure Chemicals. 1,4-Benzoquinone, 1,4-naphthoquinone, and menadione were products of Nacalai Tesque (Kyoto). 2-Methyl-1,4-benzoquinone was purchased from Wako Pure Chemicals. NADPH was obtained from Oriental Yeast (Tokyo). Superoxide dismutase (human recombinant Cu,Zn-superoxide dismutase) was a gift from Nippon Kayaku (Tokyo), and cytochrome *c* was

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Abbreviations: RHCR, rabbit heart carbonyl reductase; 4BP, 4-benzoylpyridine.

purchased from Sigma. All other chemicals were of reagent grade.

Purification of RHCR—RHCR was purified from the cytosolic fraction of rabbit heart, with 4BP as the substrate, according to the method reported previously (*11*). The purified enzyme was confirmed to be a homogeneous protein on SDS-PAGE.

Enzyme Assay—The enzyme activity was assayed spectrophotometrically by monitoring NADPH oxidation at 340 nm. The reaction mixture consisted of 100 mM sodium potassium phosphate buffer (pH 6.0), 0.25 mM NADPH, 4BP or quinones at various concentrations, and the purified enzyme, in a total volume of 0.7 ml. The reaction was initiated by the addition of the enzyme. One unit of enzyme activity was defined as the amount causing a decrease in absorbance at 340 nm corresponding to the oxidation of 1 μmol of NADPH/min at 30°C. Protein concentrations were determined with bovine serum albumin as the standard by the method of Lowry *et al.* (*23*).

Inhibition Studies—Inhibition studies were carried out using 4BP or menadione as the substrate. Flavonoids were dissolved in methanol and then added to the reaction mixture described above. The final concentration of methanol did not exceed 2% (v/v), and this concentration did not affect the enzyme activity. The IC_{50} values (the concentrations of flavonoids required to inhibit the enzyme activity by 50%) were calculated from linear regression of no less than five points according to the method of Zhang and Das (*21*).

Molecular Conformation of Flavonoids—The energy-minimized structure of the flavonoids was calculated by means of molecular mechanics (MM2) (*24*).

Kinetic Analysis—The data were plotted according to the double-reciprocal linear transformation of the Michaelis-Menten equation. The inhibition constants, K_{ii} and K_{is}, were estimated from replots of the intercepts and slopes, respectively, of the double-reciprocal plots in the presence of quercetin.

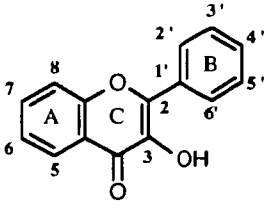
Determination of the Superoxide Anion Radical—The superoxide anion radical was determined by the method of McCord and Fridovich (*25*) using cytochrome *c*. The reaction mixture, with a final volume of 0.7 ml, consisted of 100 mM sodium potassium phosphate buffer (pH 6.0), 0.1 mM NADPH, 0.1 mM EDTA, 50 μM cytochrome *c*, and 1.0 μM RHCR. The reaction was started by the addition of 50 μM menadione. The reduction of ferricytochrome *c* (Fe^{3+}) to ferrocytochrome *c* (Fe^{2+}) in the enzyme reaction system was measured by recording the absorbance at 550 nm.

RESULTS

Structure-Activity Relationship—A structure-activity study of chemically related flavonoids (five flavonols and one flavanonol) was conducted to evaluate the structural requirements for RHCR inhibition. 4BP was used, at the concentration of 1.0 mM, as a well-known substrate of carbonyl reductases. The IC_{50} values obtained are summarized in Table I. The order of the inhibitory potencies of the flavonoids toward RHCR was kaempferol > quercetin = galangin > myricetin > morin > taxifolin. It is noteworthy that kaempferol is a more potent inhibitor of RHCR than myricetin and quercetin, which possess, respectively, pyrogallol and catechol moieties in the phenyl ring (B ring). Quercetin (IC_{50} = 10.1 ± 0.5 μM) was a more potent inhibitor of RHCR than morin (IC_{50} = 40.1 ± 4.2 μM), even though they have identical numbers of hydroxyl groups in the same positions, except for the substitution of a hydroxyl

TABLE I. **Inhibitory effects of flavonoids on RHCR.** The concentrations of 4BP and NADPH were 1.0 and 0.25 mM, respectively. The IC_{50} values are the means ± SD for three experiments. The partition coefficients (log *P*) are cited from Ref. *20*.

Flavonol



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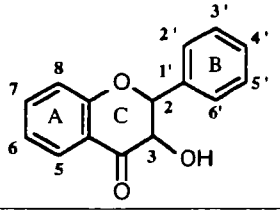

Flavonoid	Class	Hydroxylation pattern	log *P*	IC_{50} (μM)
Galangin	Flavonol	5, 7,	0.709	10.9 ± 1.0
Kaempferol	Flavonol	5, 7, 4′	0.213	5.5 ± 0.6
Quercetin	Flavonol	5, 7, 3′, 4′	−0.283	10.1 ± 0.5
Myricetin	Flavonol	5, 7, 3′, 4′, 5′	−0.779	14.5 ± 1.0
Morin	Flavonol	5, 7, 2′, 4′	−0.283	40.1 ± 4.2
Taxifolin	Flavanonol	5, 7, 3′, 4′	0.081	79.3 ± 5.1

Quercetin

Morin

Taxifolin

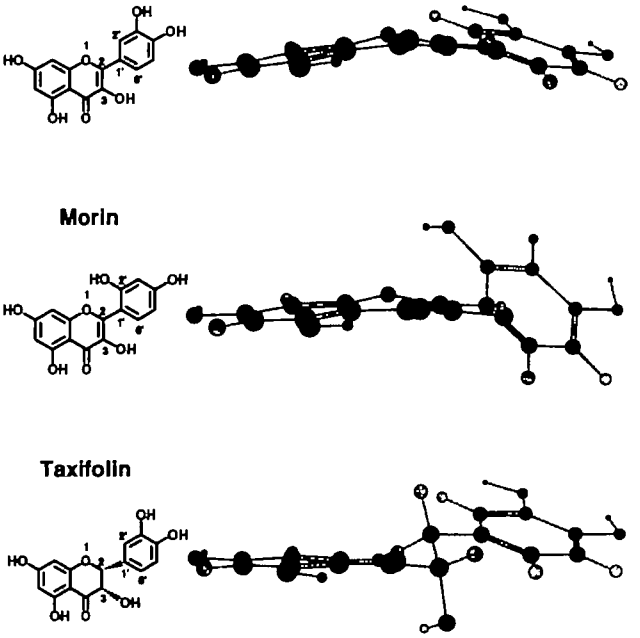

Fig. 1. **Molecular conformations of quercetin, morin and taxifolin.** The energy-minimized structures of the flavonoids are shown. The energy-minimized structures of flavonoids were calculated by means of molecular mechanics (MM2). The energy-minimized structure of taxifolin was determined as a racemic *cis* isomer.

group for the 2′- and 3′-position in the phenyl ring, as evident from the chemical structures in Table I. Furthermore, the inhibitory potency of quercetin, a flavonol, was much higher than that of taxifolin (IC_{50} = 79.3 ± 1.0 μM), a flavanonol; these two flavonoids are the same in the numbers and positions of hydroxyl groups. Since quercetin has a 2,3-double bond in the C ring but taxifolin does not, the 2,3-double bond in the C ring probably contributes to the inhibitory potencies of the flavonoids toward RHCR.

To further establish the structure-activity relationship of RHCR inhibition by flavonoids, the energy-minimized structures of flavonoids were calculated. Figure 1 shows the constructed molecular conformations of quercetin, morin and taxifolin. The dihedral angles (3-2-1′-6′) between the pyrone rings (C rings) and the phenyl rings of quercetin and morin were 33.7° and 45.5°, respectively, indicating that morin has a twisted conformation between its pyrone ring and phenyl ring as compared with quercetin. The molecular conformations of galangin, kaempferol, and myricetin were similar to that of quercetin (data not shown), and the dihedral angles between their pyrone rings and phenyl rings were 33.7–33.9°. The pyran ring (C ring) of taxifolin, unlike the pyrone rings of quercetin and morin, was puckered due to the saturation of the 2,3-double bond, which greatly influences its conformation.

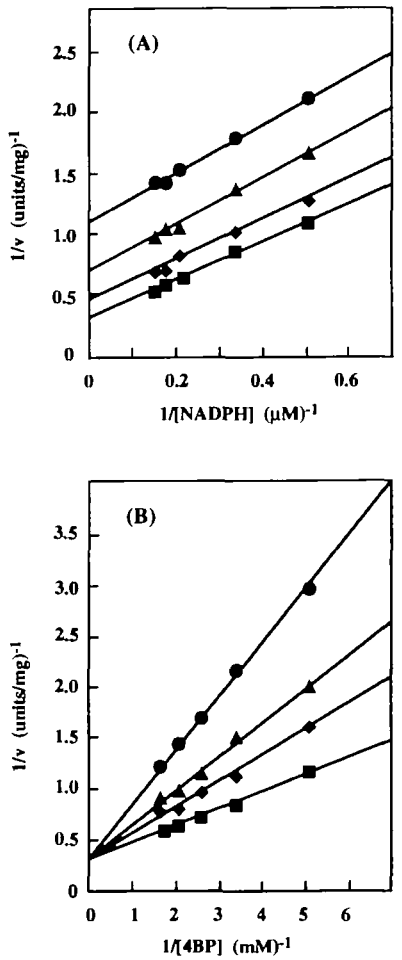

Fig. 2. **Double-reciprocal plots for the inhibition of RHCR by quercetin.** (A) The concentration of 4BP was 1.0 mM. (B) The concentration of NADPH was 0.25 mM. The concentrations of quercetin were 0 μM (■), 1.0 μM (◆), 2.0 μM (▲), and 5.0 μM (●).

Kinetic Mechanism—The inhibitory effects of five flavonoids belonging to the flavonol family on RHCR were kinetically examined. Figure 2 shows double-reciprocal plots for the inhibition of RHCR by quercetin. Quercetin was found to inhibit RHCR uncompetitively with respect to NADPH and competitively with respect to 4BP. The inhibition patterns were confirmed by comparing the values of the two inhibition constants, K_u and K_{is} (Table II). Furthermore, we attempted to elucidate the mechanism of RHCR inhibition by flavonoids other than quercetin according to the method of Yonetani and Theorell (*26*). For this method, quercetin was used as a standard inhibitor, and the values of V_0/V_i (initial velocity in the absence of inhibitor/initial velocity in the presence of quercetin or in the presence of quercetin and another flavonoid at a fixed concentration) were plotted against the concentrations of quercetin. As shown in Fig. 3A, the plots for the double-inhibition of RHCR by quercetin and galangin (5 μM) were parallel to those for the single-inhibition of RHCR by quercetin. A similar inhibition pattern was observed for the respective combinations of quercetin and kaempferol (5 μM), myricetin (15 μM), and morin (30 μM) (Fig. 3B-D). These results indicate that these five flavonoids bind at the same site on RHCR (*26*).

Substrate Specificity for Quinones—The substrate specificity of RHCR for quinones is summarized in Table III. The enzyme efficiently reduced benzoquinones (1,4-benzoquinone and 2-methyl-1,4-benzoquinone) and naphthoquinones (1,4-naphthoquinone and menadione), which are typical and relatively stable quinones. However, 9,10-anthraquinone and 9,10-phenanthrenequinone were not reduced (data not shown). When menadione at the concentration of 0.5 mM was used as the substrate, galangin was a potent inhibitor of RHCR, and the IC_{50} value (9.9 ± 1.2 μM) was the same as that obtained using 4BP at the concentration of 1.0 mM as the substrate (see Table I).

Protective Effect against the Formation of the Superoxide Anion Radical—Menadione is known to be more toxic to cultured cardiomyocytes than to skeletal muscle cells, smooth muscle cells, and hepatocytes (*15*). Thus, we examined whether or not RHCR catalyzes the redox cycling of menadione and causes the formation of the superoxide anion radical. As shown in Fig. 4, the absorbance of cytochrome *c* at 550 nm increased with the time in the presence of RHCR, NADPH, and menadione, indicating that ferricytochrome *c* was reduced to ferrocytochrome *c*. Generally, in the generation system for the superoxide anion radical, superoxide dismutase is known to inhibit 80% or more of ferricytochrome *c* reduction. However, as shown in Fig. 4, superoxide dismutase could not fully abolish the enhanced absorbance of cytochrome *c* at 550 nm. This is because the

TABLE II. **Inhibition patterns of RHCR with quercetin.** The values of K_u and K_{is} were determined from replots of the intercepts and slopes, respectively, in Fig. 2.

Varied substrate	Fixed substrate	Inhibition pattern	Inhibition constant	
			K_{ii} (μM)	K_{is} (μM)
NADPH	1.0 mM 4BP	Uncompetitive	2.17	—
4BP	0.25 mM NADPH	Competitive	—	2.15

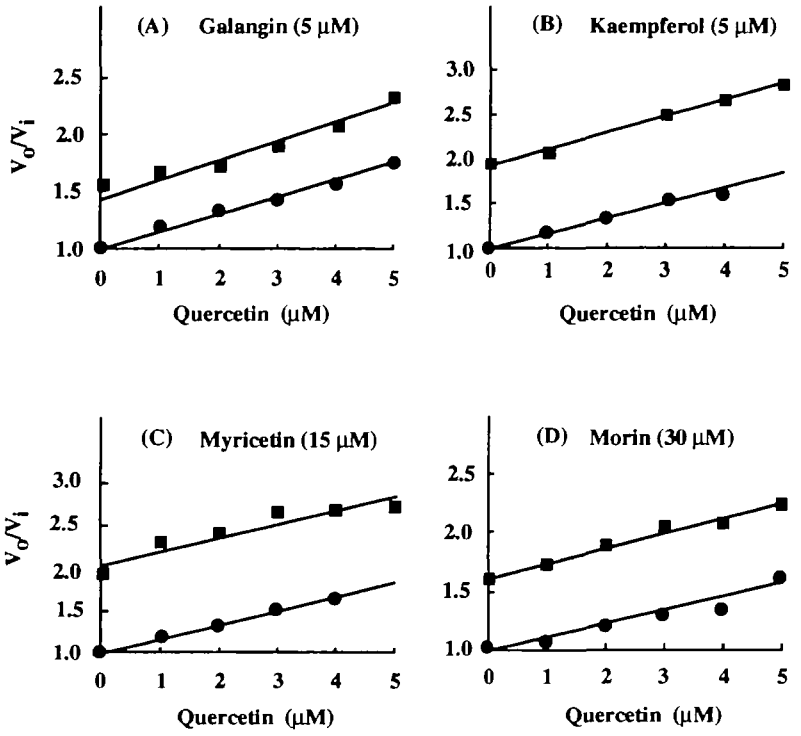

Fig. 3. **Yonetani-Theorell plots for the inhibition of RHCR by flavonoids.** The concentrations of 4BP and NADPH were 1.0 and 0.25 mM, respectively. ●, plots for single-inhibition of RHCR by quercetin; ■, plots for double-inhibition of RHCR by quercetin and another flavonoid at a fixed concentration.

TABLE III. **Substrate specificities of RHCR for quinones.** The K_m and V_{max} values of the enzyme for substrates were determined by means of least-squares linear regression of double-reciprocal plots. The concentration of NADPH was 0.25 mM. All values are the means ± SD for three experiments.

Quinone	K_m (mM)	V_{max} (units/mg)
1,4-Benzoquinone	1.13 ± 0.17	143.8 ± 7.6
2-Methyl-1,4-benzoquinone	0.57 ± 0.07	71.2 ± 7.1
1,4-Naphthoquinone	0.54 ± 0.10	27.1 ± 6.6
Menadione	0.32 ± 0.06	7.6 ± 1.2

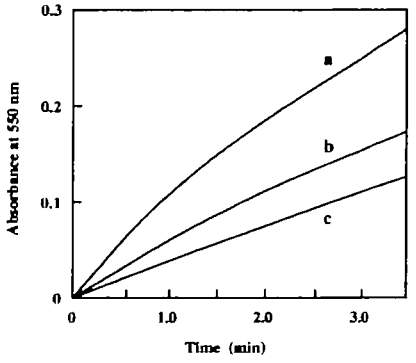

Fig. 4. **Menadione-mediated reduction of cytochrome *c* in the reaction system of RHCR.** Superoxide dismutase (100 units) or galangin (10 μM) was added to the reaction system. The reaction was started by the addition of 50 μM menadione. a, complete reaction system; b, complete reaction system + superoxide dismutase; c, complete reaction system + galangin.

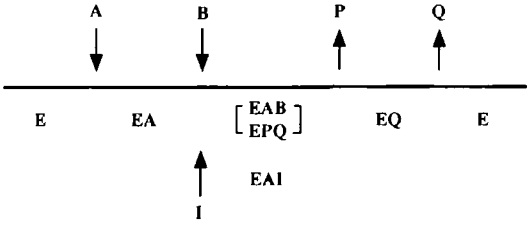

Fig. 5. **Proposed kinetic mechanism for RHCR.** E, enzyme (RHCR); A, NADPH; B, 4BP; P, reduction product of 4BP (α-phenyl-4-pyridylmethanol); Q, NADP; I, inhibitor (quercetin).

semiquinone produced in this reaction system also reduces ferricytochrome *c*, as has been pointed out by Winterbourn (27). Similar limited inhibition of ferricytochrome *c* reduction by superoxide dismutase has been observed for the ζ-crystallin/NADPH:quinone oxidoreductase system (28). On the other hand, galangin at the concentration of 10 μM decreased the enhanced absorbance of cytochrome *c* at 550 nm to about one-half (Fig. 4).

DISCUSSION

In the present study, we demonstrated that the numbers and positions of hydroxyl group(s) in the phenyl rings (B rings) of flavonoids and the existence of a 2,3-double bond in the C ring are important for their RHCR inhibition. Quercetin and morin have the same lipophilic parameters (see the log *P* values in Table I). However, the inhibitory potency of morin toward RHCR was much lower than that of quercetin. The stereochemical characteristics of the flavonoids observed in this study showed that morin has a twisted conformation between the pyrone ring and the phenyl ring as compared with quercetin. Furthermore, the X-ray crystal structure analysis of these two flavonoids has provided evidence that the phenyl ring of quercetin is co-

planar with the pyrone ring, whereas the molecular conformation of morin is twisted between the pyrone ring and the phenyl ring (*29*, *30*). Based on these structural analysis data, we conclude that the stereochemical characteristics is a factor determining the inhibitory potencies of flavonoids toward RHCR. This is supported by the fact that taxifolin, a flavanonol, is the poorest inhibitor of RHCR, since the benzopyran ring (A and C rings) of flavanonols, unlike the benzopyrone ring (A and C rings) of flavonols, is not planar. Ferriola *et al.* (*19*) have also reported that the inhibitory potencies of flavonoids toward protein kinase C are characterized by a planar benzopyrone ring with a coplanar phenyl ring.

Galangin, kaempferol and myricetin have molecular conformations similar to that of quercetin, but these flavonoids including quercetin exhibited different inhibitory activities toward RHCR. The inhibitory potencies of kaempferol, quercetin and myricetin toward RHCR decreased with increasing numbers of hydroxyl groups in the phenyl ring, suggesting the existence of a hydrophobic region in the flavonoid binding site of RHCR. However, the inhibitory potency of galangin, which has the most lipophilic nature (see the log *P* values in Table I), was found to be lower than that of kaempferol. Although the reason for this discrepancy is not yet clear, the scavenging or antioxidative effects of flavonoids may play a role in such inhibition. For example, myricetin with a pyrogallol moiety has been reported to be the most potent inhibitor of succinoxidase since it acts as a scavenger of active oxygens or as an antioxidant (*18*). We propose the possibility that in addition to the stereochemical characteristics, the lipophilicity and the scavenging or antioxidative effects of flavonoids complicate the structure-activity relationship of their inhibitory effects on RHCR.

Our previous paper (*11*) provided evidence that the reduction of 4BP catalyzed by RHCR follows an ordered Bi Bi mechanism, in which NADPH binds to the enzyme first and NADP leaves the enzyme last. In this study, quercetin was found to inhibit RHCR uncompetitively with respect to NADPH and competitively with respect to 4BP. These modes of RHCR inhibition, based on the rule of the ordered Bi Bi mechanism in the enzyme reaction, lead us to conclude that quercetin selectively binds to the enzyme-NADPH binary complex (*31*, *32*), as shown in Fig. 5. Quercetin probably competes with 4BP at the substrate-binding site of RHCR. Furthermore, the results of double-inhibition experiments (*26*) suggested that galangin, kaempferol, myricetin and morin also interact with the substrate-binding site of RHCR. Most recently, we reported that fatty acids such as myristic acid and oleic acid strongly inhibit RHCR, but that myristic acid at low concentrations inhibits RHCR uncompetitively with respect to both NADPH and 4BP (*33*). Myristic acid appeared to inhibit RHCR by interacting with a binding site other than its co-enzyme and substrate-binding sites.

We revealed in this study that RHCR efficiently reduces benzoquinones and naphthoquinones, and functions as a quinone reductase. Carbonyl reductase is thought to catalyze the two-electron reduction of quinones to hydroquinones (*16*, *17*). In the liver, the two-electron reduction of quinones by carbonyl reductase is regarded as a detoxication pathway since hydroquinones can serve as substrates for glucuronidation and sulfation reactions which terminate redox cycling. However, Brown *et al.* (*34*) have pointed out that when these secondary reactions become limiting, the autoxidation of hydroquinones contributes to the generation of reactive oxygen species. In the heart, the presence of enzymes responsible for secondary reactions such as glucuronidation and sulfation is unknown. Thus, it is possible that the hydroquinones produced by RHCR are accumulated in the heart and that their autoxidation induces the formation of the superoxide anion radical (*11*), suggesting that quinones exert a toxic effect on the heart through the process of their redox cycling catalyzed by RHCR.

It has been reported that galangin, unlike quercetin and myricetin, has little ability to act as an antioxidant (*35–37*). However, the present study demonstrated that galangin prevent the formation of the superoxide anion radical in the presence of RHCR, NADPH and menadione. It should be noted that galangin at the concentration of 10 μM, which corresponds to the IC_{50} value (10.9 ± 1.0 μM) of RHCR inhibition (see Table I), can decrease the enhanced absorbance of cytochrome *c* at 550 nm to about one-half. Galangin probably prevents the formation of the superoxide anion radical by inhibiting RHCR, that is, by decreasing the production of menadiol, which is a two-electron reduction product of menadione. Kaempferol, quercetin and myricetin were also potent inhibitors of RHCR. Flavonoids may be useful compounds for suppressing the cardiotoxicity of quinones by inhibiting RHCR, although the details of their gastrointestinal absorption and tissue distribution after oral administration remain to be clarified.

Our preliminary study has revealed that 4BP is efficiently reduced not only in the cytosolic fraction of rabbit heart, but also in those of pig, rat, and guinea pig hearts, and that carbonyl reductase purified from pig heart, as well as RHCR, is a tetrameric enzyme and functions as a quinone reductase. It is interesting that carbonyl reductase is widely distributed in the hearts of mammalian species including the rabbit. We are currently examining the inhibitory effects of various flavonoids on the pig heart carbonyl reductase and its catalytic properties as to quinones.

REFERENCES

1. Felsted, R.L. and Bachur, N.R. (1980) Mammalian carbonyl reductases. *Drug Metab. Rev.* **11**, 1–60
2. Jakoby, W.B. and Ziegler, D.M. (1990) The enzymes of detoxication. *J. Biol. Chem.* **265**, 20715–20718
3. Maser, E. (1995) Xenobiotic carbonyl reduction and physiological steroid oxidoreduction. The pluripotency of several hydroxysteroid dehydrogenases. *Biochem. Pharmacol.* **49**, 421–440
4. Ikeda, M., Hattori, H., and Ohmori, S. (1984) Properties of NADPH-dependent carbonyl reductases in rat liver cytosol. *Biochem. Pharmacol.* **33**, 3957–3961
5. Usui, S., Hara, A., Nakayama, T., and Sawada, H. (1984) Purification and characterization of two forms of microsomal carbonyl reductase in guinea pig liver. *Biochem. J.* **223**, 697–705
6. Imamura, Y., Higuchi, T., Nozaki, Y., Sugino, E., Hibino, S., and Otagiri, M. (1993) Purification and properties of carbonyl reductase from rabbit kidney. *Arch. Biochem. Biophys.* **300**, 570–576
7. Wermuth, B. (1981) Purification and properties of an NADPH-dependent carbonyl reductase from human brain. *J. Biol. Chem.* **256**, 1206–1213
8. Nakayama, T., Hara, A., and Sawada, H. (1982) Purification and characterization of a novel pyrazole-sensitive carbonyl reductase in guinea pig lung. *Arch. Biochem. Biophys.* **217**, 564–573

9. Oritani, H., Deyashiki, Y., Nakayama, T., Hara, A., Sawada, H., Matsuura, K., Bunai, Y., and Ohya, I. (1992) Purification and characterization of pig lung carbonyl reductase. *Arch. Biochem. Biophys.* **292**, 539–547
10. Iwata, N., Inazu, N., and Satoh, T. (1989) The purification and properties of NADPH-dependent carbonyl reductases from rat ovary. *J. Biochem.* **105**, 556–564
11. Imamura, Y., Migita, T., Otagiri, M., Choshi, T., and Hibino, S. (1999) Purification and catalytic properties of a tetrameric carbonyl reductase from rabbit heart. *J. Biochem.* **125**, 41–47
12. Wermuth, B., Platts, K.L., Seidel, A., and Oesch, F. (1986) Carbonyl reductase provides the enzymatic basis of quinone detoxication in man. *Biochem. Pharmacol.* **35**, 1277–1282
13. O'Brien, P. (1991) Molecular mechanisms of quinone cytotoxicity. *Chem. Biol. Interact.* **80**, 1–41
14. Monks, T.J., Hanzlik, R.P., Cohen, G.M., Ross, D., and Graham, D.G. (1992) Quinone chemistry and toxicity. *Toxicol. Appl. Pharmacol.* **112**, 2–16
15. Tzeng, W.F., Chiou, T.J., Lee, J.L., and Chen, Y.H. (1995) The role of lipid peroxidation in menadione-mediated toxicity in cardiomyocytes. *J. Mol. Cell Cardiol.* **27**, 1999–2008
16. Jarabak, J. (1991) Polycyclic aromatic hydrocarbon quinone-mediated oxidation reduction cycling catalyzed by a human placental NADPH-linked carbonyl reductase. *Arch. Biochem. Biophys.* **291**, 334–338
17. Jarabak, J. and Harvey, R.G. (1993) Studies on three reductases which have polycyclic aromatic hydrocarbon quinones as substrates. *Arch. Biochem. Biophys.* **303**, 394–401
18. Hodnick, W.F., Milosavljevic, E.B., Nelson, J.H., and Pardini, R.S. (1988) Electrochemistry of flavonoids: Relationship between redox potentials, inhibition of mitochondrial respiration, and production of oxygen radicals by flavonoids. *Biochem. Pharmacol.* **37**, 2607–2611
19. Ferriola, P.C., Cody, V., and Middleton, E., Jr. (1989) Protein kinase C inhibition by plant flavonoids: Kinetic mechanisms and structure-activity relationship. *Biochem. Pharmacol.* **38**, 1617–1624
20. Elliott, A.J., Scheiber, S.A., Thomas, C., and Pardini, R.S. (1992) Inhibition of glutathione reductase by flavonoids: A structure-activity study. *Biochem. Pharmacol.* **44**, 1603–1608
21. Zhang, K. and Das, N.P. (1994) Inhibitory effects of plant polyphenols on rat liver glutathione S-transferases. *Biochem. Pharmacol.* **47**, 2063–2068
22. Zhai, S., Dai, P., Friedman, F.K., and Vestal, R.E. (1998) Comparative inhibition of human cytochromes P450 1A1 and 1A2 by flavonoids. *Drug Metab. Dispos.* **26**, 989–992
23. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
24. Allinger, N.L. (1988) Molecular mechanics calculations on conjugated nitrogen-containing heterocycles. *J. Am. Chem. Soc.* **110**, 2050–2055
25. McCord, J.M. and Fridovich, I. (1969) Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **244**, 6049–6055
26. Yonetani, T. and Theorell, H. (1964) Studies on liver alcohol dehydrogenase complexes III. Multiple inhibition kinetics in the presence of two competitive inhibitors. *Arch. Biochem. Biophys.* **106**, 243–251
27. Winterbourn, C.C. (1981) Cytochrome c reduction by semiquinone radicals can be indirectly inhibited by superoxide dismutase. *Arch. Biochem. Biophys.* **209**, 159–167
28. Rao, P.V., Krishna, C.M., and Zigler, J.S., Jr. (1992) Identification and characterization of the enzymatic activity of ζ-crystallin from guinea pig lens: A novel NADPH:quinone oxidoreductase. *J. Biol. Chem.* **267**, 96–102
29. Cody, V. (1988) Crystal and molecular structures of flavonoids in *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties* (Cody, V., Middleton, E., Jr., Harborne, J.B., and Beretz, A., eds.) pp. 29–44, Alan R. Liss, Inc., New York
30. Rossi, M., Rickles, L.F., and Halpin, W.A. (1986) The crystal and molecular structure of quercetin: A biologically active and naturally occurring flavonoid. *Bioorg. Chem.* **14**, 55–69
31. Svensson, S., Strömberg, P., and Höög, J.-O. (1999) A novel subtype of class II alcohol dehydrogenase in rodents: Unique Pro^{47} and Ser^{182} modulates hydride transfer in the mouse enzyme. *J. Biol. Chem.* **274**, 29712–29719
32. Imamura, Y., Koga, T., Migita, T., Ryu, A., Otagiri, M., Nozawa, M., and Akita, H. (1997) Characterization of acetohexamide reductases purified from rabbit liver, kidney, and heart: Structural requirements for substrates and inhibitors. *J. Biochem.* **121**, 705–710
33. Imamura, Y., Migita, T., Anraku, M., and Otagiri, M. (1999) Inhibition of rabbit heart carbonyl reductase by fatty acids. *Biol. Pharm. Bull.* **22**, 731–733
34. Brown, P.C., Dulik, D.M., and Jones, T.W. (1991) The toxicity of menadione (2-methyl-1,4-naphthoquinone) and two thioether conjugates studied with isolated renal epithelial cells. *Arch. Biochem. Biophys.* **285**, 187–196
35. 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 antioxidant activity of flavonoids. *Free Rad. Biol. Med.* **20**, 331–342
36. Miura, Y.H., Tomita, I., Watanabe T., Hirayama, T., and Fukui, S. (1998) Active oxygens generation by flavonoids. *Biol. Pharm. Bull.* **21**, 93–96
37. van Acker, S.A.B.E., van Balen, G.P., van den Berg, D.-J., Bast, A., and van der Vijgh, W.J.F. (1998) Influence of iron chelation on the antioxidant activity of flavonoids. *Biochem. Pharmacol.* **56**, 935–943