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Mechanism-Based Inactivation of COX-1 by Red Wine *m*-Hydroquinones: A Structure−Activity Relationship Study

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Resveratrol (**1**) is a *m*-hydroquinone found in red wine, which has antiinflammatory, cardiovascular protective (antiplatelet), and cancer chemopreventive properties. It is a potent peroxidase-dependent mechanism-based inactivator of COX-1, a desired target for antiplatelet agents, and has no similar effect on COX-2. Much attention has focused on resveratrol (**1**) as being the sole agent responsible for the cardioprotective effects associated with red wine consumption (commonly known as the "French paradox"). In this study we show that other red wine constituents, namely, the catechins (**2**, **3**) and epicatechins (**4**, **5**), act as peroxidase mediated mechanism-based inactivators of COX-1 but not of COX-2. Structure−activity relationships identify these agents as being as effective as resveratrol with respect to their ability to specifically inactivate COX-1. We show that resorcinol (**6**) is the minimum structure necessary for mechanism-based inactivation of COX-1. These findings imply that resveratrol is not the sole agent responsible for the antiplatelet activity of red wine and suggest that all dietary *m*-hydroquinones should be examined for cardioprotective effects.

Resveratrol (**1**) (3,5,4′-trihydroxy-*trans*-stilbene; Scheme 1) is a natural product present at concentrations up to 100 μM in red wines.[1] It is reported to have antiinflammatory, cardiovascular protective, and cancer chemopreventive properties and was shown to target prostaglandin H_2 synthases (COX-1 and COX-2).[2,3] Its presence in red wine and its pharmacological properties identify it as one putative agent responsible for the cardioprotective (antiplatelet) effects observed with the increased consumption of red wine (commonly known as the "French paradox").[4] Much attention has focused on resveratrol as the sole agent responsible for the "French paradox"; however, the physiological relevance of this compound has remained questionable since it has poor pharmacokinetic parameters including low bioavailability and rapid clearance from the plasma.[5−7]

The COX-1 and COX-2 targets for resveratrol catalyze the first committed steps in the synthesis of all vasoactive prostaglandins (PGs). They convert arachidonic acid (AA) to PGH_2 by two sequential reactions that occur at spatially distinct active sites on the enzymes.[8−10] PGs are local mediators of vascular homeostasis; for example, thromboxane A_2 (TxA_2) is a potent vasoconstrictor and platelet aggregator synthesized in activated platelets by COX-1, while prostacyclin (PGI_2) is an antiplatelet aggregator and potent vasodilator synthesized in the vascular endothelial cells by COX-2.[10−13] Therefore, selective inhibition of COX-1 offers a viable mechanism for cardioprotective (antiplatelet) agents, which can act by tilting the TxA_2−PGI_2 balance in favor of PGI_2.[14,15] It is through this mechanism that low-dose aspirin exerts its cardioprotective effects.[16−19]

We showed that resveratrol (**1**) is a potent mechanism-based inactivator of COX-1, an enzyme involved in maintaining vascular homeostasis. In contrast, resveratrol acted only as a reducing co-substrate for COX-2 and reduced the heme Fe to its resting state for the next round of peroxidase

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Scheme 1. Structures of *m*-Hydroquinones Present in Red Wine

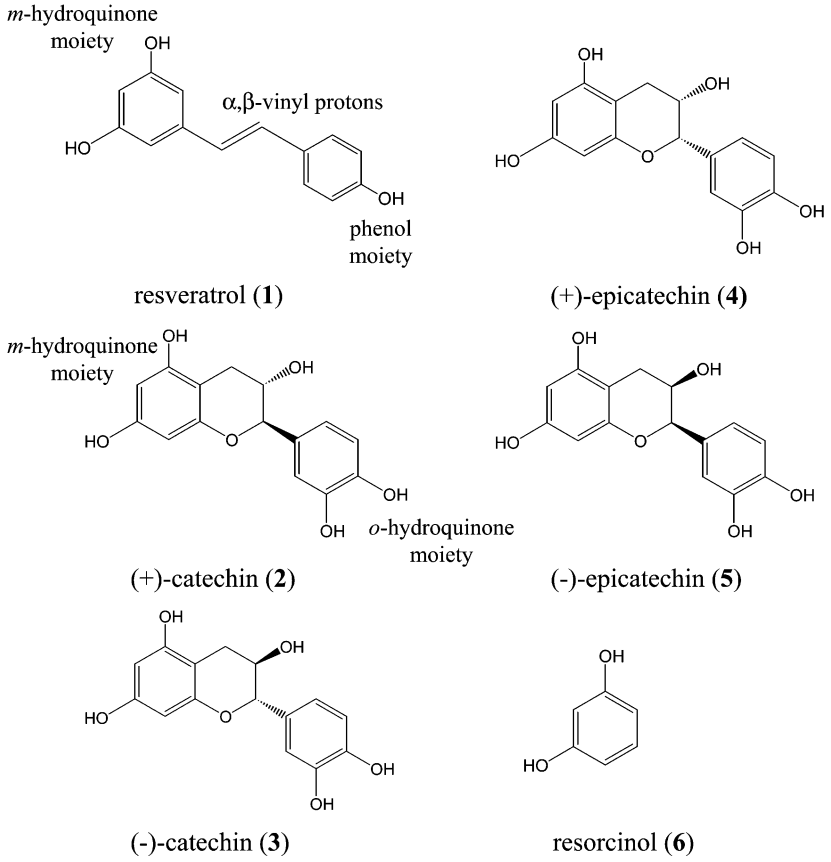


Table 1. SAR Analysis of Red Wine *m*-Hydroquinones on COX-1

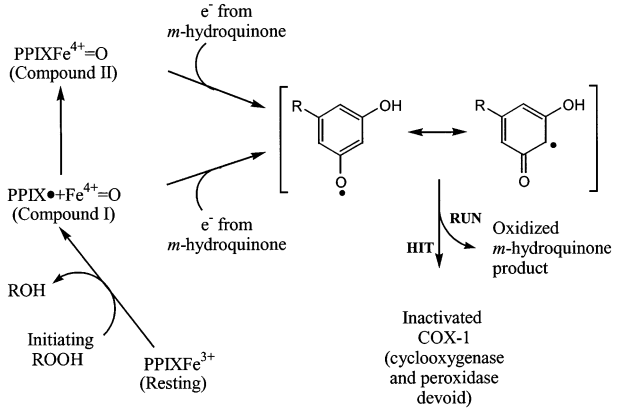
analogue	IC_{50} peroxidase (μM)	IC_{50}[a] cyclooxgenase (μM)	K_d (μM)	% analogue oxidized (RP-HPLC)	mode of action[b] on COX-1	mode of action on COX-2
1	2.8 ± 0.6	67.3 ± 18.9	11.7 ± 1.8	47	inactivator	co-reductant
2	4.1 ± 0.4	20.2 ± 3.2	40.7 ± 3.1	7	inactivator	co-reductant
3	5.3 ± 0.2	30.8 ± 2.7	48.7 ± 4.1	8	inactivator	co-reductant
4	2.5 ± 0.3	12.8 ± 1.3	25.1 ± 5.8	5	inactivator	co-reductant
5	2.0 ± 0.1	12.2 ± 1.3	25.9 ± 6.5	5	inactivator	co-reductant
6	3.6 ± 0.6	30.8 ± 24.2	86.9 ± 29.5	5	inactivator	co-reductant

[a] Values elevated because 1 mM phenol is present in the assay and no peroxide co-substrate is added. [b] $k_{inact} > 0.025\ s^{-1}$ and $t_{1/2} < 25$ s for all compounds when tested at 5 μM in the TMPD peroxidase assay.

catalysis. Inactivation of COX-1 by resveratrol occurred at low micromolar concentrations, it had an absolute requirement for a peroxide substrate, it occurred at the peroxidase active site, and it resulted in the complete loss of PG synthesis by this isoform. Furthermore, mechanism-based inactivation proceeded via a "hit-and-run" mechanism in which COX-1 was not covalently modified (Scheme 2). These findings were consistent with catalytic mechanisms, which require the peroxidase activity to initiate the cyclooxygenase activity.[20]

Several observations suggest that resveratrol (**1**) may not be the sole component responsible for the cardioprotective (antiplatelet) effects of red wine. First, the average drinker absorbs amounts of resveratrol that would yield submicromolar plasma concentrations, which may be insufficient to inactivate COX-1.[5,6] Second, irreversible inactivation of COX-1 by resveratrol had an absolute requirement for a peroxide substrate and is prevented by a co-reductant.[20] Thus, the cardioprotective effects of resveratrol may be limited by pharmacokinetics, the prevailing low peroxide tone of the resting platelet, and the co-reductant tone of the resting platelet.

Scheme 2. Proposed Mechanism for the Inactivation of COX-1 by *m*-Hydroquinones



We now show that other *m*-hydroquinones in red wine [e.g., catechins (**2**, **3**) and epicatechins (**4**, **5**); Scheme 1] also act as peroxidase-dependent mechanism-based inactivators of COX-1. We present structure−activity relationship (SAR) data that identify these agents as being as effective

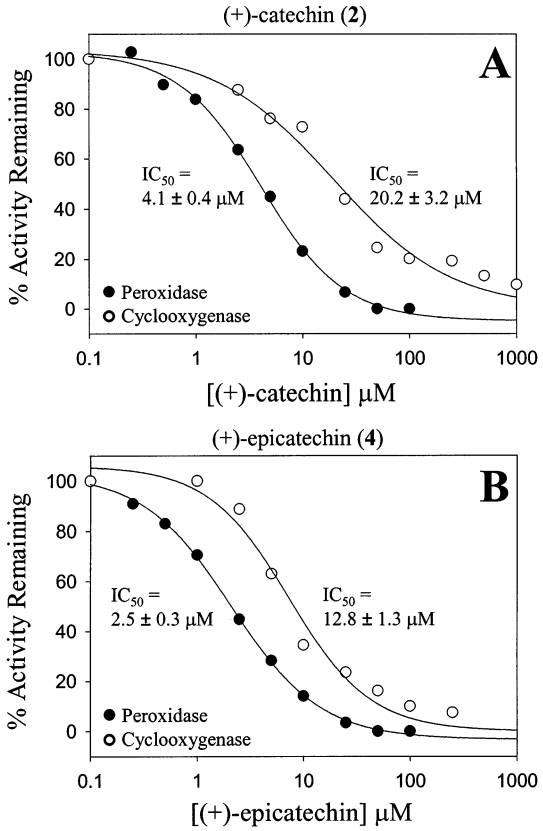


Figure 1. Dose−response curves for the inhibition of COX-1 by *m*-hydroquinones. IC_{50} values were measured for either the peroxidase activity, using the TMPD assay, or the cyclooxygenase activity, using the oxygen consumption assay, in the presence of increasing amounts of analogue. Analogue (0.1−1000 μM), FePPIX (2 μM), and COX-1 were mixed in 100 mM Tris-HCl (pH 8.0) supplemented with either 80 μM TMPD (peroxidase assay) or 1 mM phenol (cyclooxygenase assay). Reactions were initiated by the addition of either 300 μM H_2O_2 (peroxidase assay) or 150 μM AA (cyclooxygenase assay). Representative dose−response curves for the peroxidase and cyclooxygenase activities are shown for (A) (+)-catechin and (B) (+)-epicatechin.

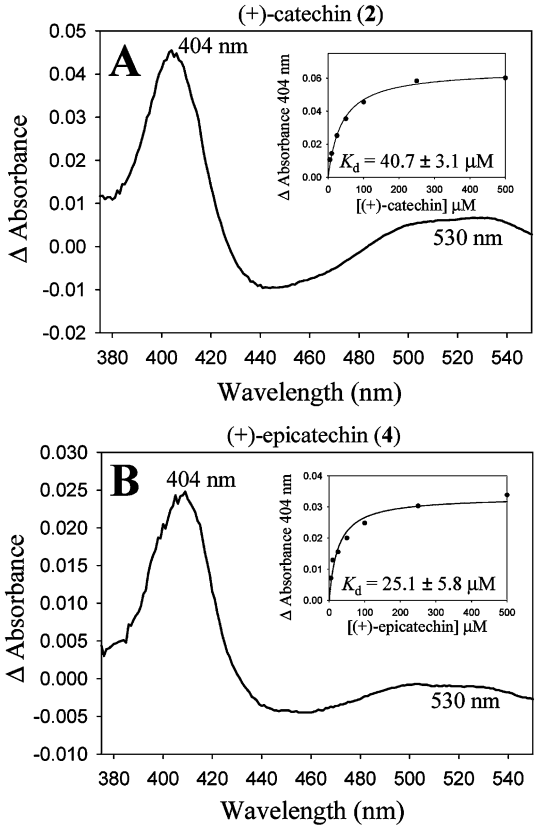


Figure 2. Difference spectra for the formation of an enzyme·drug complex and measurement of K_d. COX-1 (2.5 μM) plus FePPIX (5 μM) and either DMSO or analogue (10−500 μM) were mixed in 100 mM Tris-HCl (pH 8.0), and absorbance spectra were collected from 375 to 550 nm on a diode array spectrophotometer. Difference spectra were generated by subtracting the absorbance spectrum of solvent-treated enzyme from that of analogue-treated enzyme. K_d for analogue binding to COX-1 was determined by monitoring Δabsorbance at 404 nm while the concentration of analogue was incrementally increased (10−500 μM). Representative difference spectra obtained using 100 μM (+)-catechin and 100 μM (+)-epicatechin are shown along with their binding isotherms (insets) in A and B respectively.

as resveratrol (**1**) with respect to their ability to inactivate COX-1 and identify the minimum structure required for COX-1 inactivation. These compounds are more concentrated in red wine and should yield higher plasma concentrations.[21,22] We hypothesize that the cardioprotective (antiplatelet) effects of red wine may result from the action of a mixture of *m*-hydroquinones on COX-1.

Results and Discussion

Interactions of Red Wine *m*-Hydroquinones with COX-1. A series of *m*-hydroquinones (Scheme 1), present in red wine, were examined for their ability to inhibit PG synthesis by COX-1 through a mechanism-based event. For each compound, the IC_{50} value for peroxidase activity, the IC_{50} value for cyclooxygenase activity, the K_d of the enzyme·drug complex, the percent oxidized by the peroxidase activity, and the mode of action on COX-1 and COX-2 were determined (Table 1). Consistent with our previous findings, all *m*-hydroquinone-containing compounds were potent inhibitors of both the peroxidase and cyclooxygenase reactions of COX-1 (Table 1; see Figure 1 for representative data). In every case, the analogues appeared to be more potent inhibitors of the peroxidase activity [IC_{50} values between 2.0 μM (**5**) and 5.3 μM (**3**)] than of the cyclooxygenase activity [IC_{50} values between 12.2 μM (**5**) and 67.3 μM (**1**)]. Reasons for this apparent difference in potency will be discussed later.

Incubation of holo-COX-1 with each analogue (in the absence of substrates) yielded changes in the absorbance spectrum of the enzyme. These changes were identified by difference spectroscopy and are defined by an increase in intensity of the Soret band at 404 nm and the appearance of a new chromophore at 530 nm (see Figure 2 for representative data). The increase in absorbance of the Soret band with COX-1 was dependent on the concentration of the analogue and was saturable. This allowed a K_d to be determined for each analogue (Table 1; see Figure 2 insets for representative data). These K_d values ranged from 11.7 μM (**1**) to 86.9 μM (**6**).

Evidence that each analogue was oxidized by the peroxidase activity of COX-1 was provided by an endpoint RP-HPLC analysis. The enzymatic depletion of each analogue had an obligatory requirement for peroxide substrate (H_2O_2). COX-1 was able to oxidize 47% (23.5 nmol) of resveratrol (**1**) in the assay system, but only 5−8% (2.5−4 nmol) of the other analogues (**2**−**6**) were oxidized (Table 1; see Figure 3 for RP-HPLC separation method).

Preincubation studies of COX-1 with each analogue and peroxide co-substrate (H_2O_2) were revealing (see Figure 4). First, *m*-hydroquinones alone had no effect on the enzyme during a 5 min preincubation period (control). However, under conditions in which the analogue was oxidized (ca. in the presence of H_2O_2) there was a significant increase in the amount of enzyme inactivation observed. Less than

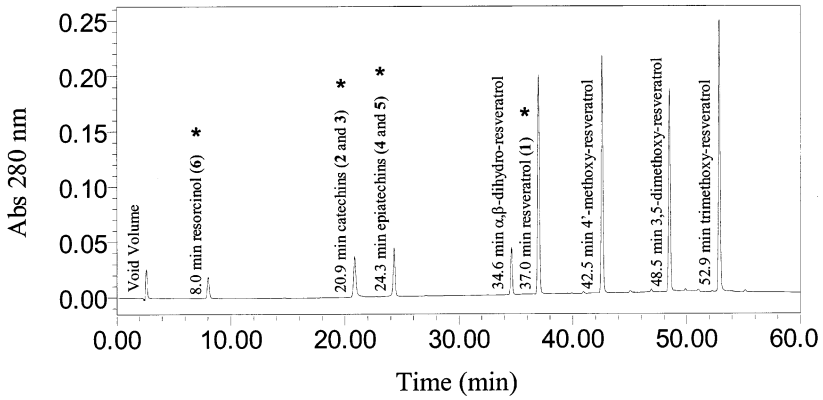


Figure 3. RP-HPLC method used to separate red wine *m*-hydroquinones. A mixture of *m*-hydroquinones, which contained equal amounts of analogues **1**−**6** as well as the methoxy-resveratrol analogues, was prepared in DMSO. An aliquot containing 2.5 nmol of each analyte was injected onto a Waters Xterra RP_{18} column (3.5 μm; 4.6 × 150 mm) equilibrated with solvent A (10% methanol in water) at a flow rate of 0.75 mL/min. Beginning at 10 min, a linear gradient was run to solvent B (80% methanol in water) over 40 min, and isocratic flow was maintained at solvent B for an additional 10 min. The absorbance of the eluant was monitored at 280 nm. *Indicates the analogues examined in this study.

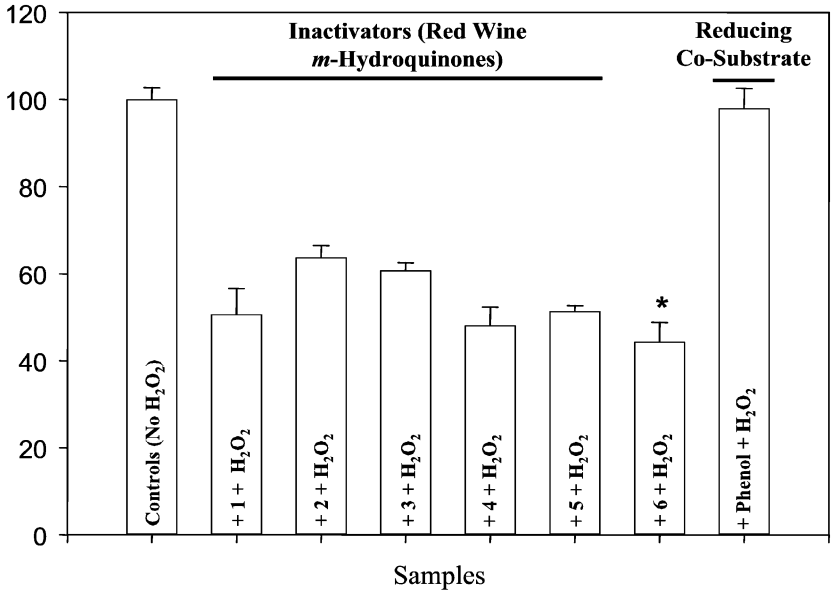


Figure 4. Mechanism-based inactivation of COX-1 by red wine *m*-hydroquinones. Stoichiometric quantities of COX-1 (10 μM) were preincubated with mixtures of 100 μM H_2O_2 and 100 μM analogue in 100 mM Tris-HCl (pH 8.0) supplemented with 10 μM FePPIX for 5 min at 25 °C. Preincubations were initiated with H_2O_2 (or H_2O when peroxide was not a reagent). Immediately following preincubation, the samples were diluted 200-fold into the peroxidase assay. Activity measurements were corrected for resveratrol carryover according to IC_{50} curves, and the percent activity remaining was computed with respect to an enzyme control. Less than 20% of the observed inactivation can be attributed to enzyme self-inactivation. *Indicates the minimum SAR for inactivation.

20% of the observed inactivation could be attributed to enzyme self-inactivation. By contrast, a prototypical reducing co-substrate, phenol, prevented peroxidase-dependent inactivation. These findings identify the catechins (**2** and **3**) and epicatechins (**4** and **5**) as peroxidase-dependent mechanism-based inactivators of COX-1 and are consistent with our previous studies on resveratrol (**1**) and its methoxy analogues.[20] Furthermore, resorcinol (**6**) was identified as the minimum structure necessary for mechanism-based inactivation of COX-1. With respect to COX-2, these analogues acted only as reducing co-substrates (e.g., phenol), indicating that they were selective inactivators for COX-1.

Structure−Activity Relationships. With respect to each analogue, there was a 5−20-fold difference in potency between the IC_{50} value for the peroxidase activity and the IC_{50} value for the cyclooxygenase activity. In every case, the IC_{50} value for the cyclooxygenase activity was higher. It is possible to account for this difference as follows: Inactivation was dependent on the peroxidase activity, and it was therefore sensitive to both the level of co-reductant and the peroxide concentration in the assay system.[20] In the cyclooxygenase assay, co-reductant tone is high (1 mM phenol), and no peroxide co-substrate is added to the assay. Under these conditions, the inactivation of COX-1 will be less. In the peroxidase assay, co-reductant tone is low (80 μM TMPD) and peroxide concentration is high (300 μM H_2O_2). Under these conditions, the inactivation of the peroxidase activity of COX-1 is more favored. Therefore, the analogues appear less potent against the cyclooxygenase activity then the peroxidase activity of COX-1.

The spectroscopically determined K_d values gave insight into the specificity of the COX-1 peroxidase active site. For example, it was clear that the COX-1 peroxidase active site prefers a substituted or bicyclic *m*-hydroquinone to an unsubstituted ligand since resorcinol (**6**) had the highest K_d value (86.9 μM) compared to the other analogues (**1**−**5**). Furthermore, the *trans*-stilbene scaffold was preferred to the flavinol scaffold, as evident by resveratrol (**1**), which had a lower K_d value (11.7 μM) than the catechins (**2**, **3**) and epicatechins (**4**, **5**) (25.1−48.7 μM). Within the flavinol class, K_d values for the epicatechins (**4**, **5**) were lower than

the catechins (**2**, **3**), suggesting that there was a preference for a *cis* hydroxyl and *o*-hydroquinone configuration over a *trans*-configuration with respect to the oxane ring.

Binding Orientation and Analogue Bifunctionality. We have previously shown that all of the hydroxyl groups on resveratrol (**1**) could be oxidized by COX-1 in the presence of a peroxide co-substrate (H_2O_2), indicating that this fairly symmetrical molecule can enter the peroxidase active site in two orientations; that is, the *m*-hydroquinone or phenol moiety can be proximal to the heme cofactor. With respect to resveratrol, oxidation of the *m*-hydroquinone moiety leads to enzyme inactivation and oxidation of the phenol moiety leads to co-reduction. Therefore resveratrol is a bifunctional molecule with respect to its ability to act as both a mechanism-based inactivator and reducing co-substrate for COX-1. This bifunctionality allows large amounts of resveratrol to be oxidized by COX-1 before the enzyme is completely inactivated (47%, 23.5 nmol). In this manner, there is an equal probability associated with resveratrol acting as either an inactivator or co-reductant.[20]

The catechins (**2**, **3**) and epicatechins (**4**, **5**) also contain functionally opposing moieties, namely, the *m*-hydroquinone (inactivator) and *o*-hydroquinone (co-reductant) moiety. However, only small amounts of these analogues were oxidized prior to COX-1 inactivation (5−8%, 2.5−4 nmol). These findings suggest that the enzyme preferentially oxidized only the *m*-hydroquinone moiety, as was the case for resorcinol (**6**). In this manner, the catechins (**2**, **3**) and epicatechins (**4**, **5**) have a specific binding orientation; namely, the *m*-hydroquinone moiety is bound proximal to the heme cofactor. This preferred binding mode predicts that these analogues will be more efficient COX-1 inactivators, since this binding mode prevents the analogues from acting as co-reductants.

Our assays were insufficient to provide accurate estimates of k_{inact} and $K_{i\ inact}$ for analogues **2**−**6**. Inactivation occurred so rapidly ($t_{1/2}$ < 25 s) that the use of preincubation/dilution assays to perform kinetic analysis was precluded. Furthermore, the steady-state TMPD assay previously used to characterize the inactivation kinetics of resveratrol (**1**) and its methoxy analogues could not be employed since k_{obs} (s^{-1}) was so rapid that it was not possible to fit the data to a Kitz−Wilson analysis.[20,23] For these reasons, the bimolecular rate constants for inactivation of COX-1 by analogues **2**−**6** ($k_{inact}/K_{i\ inact}$) could not be reported. On the basis of the SAR data that were attained, one would predict that analogues **2**−**6** would have a partition ratio much less than 22, which was reported for resveratrol, since oxidation of these analogues is less and inactivation is more rapid.[20] Therefore, analogues **2**−**6** are more efficient inactivators of COX-1 than resveratrol.

Pharmacology. It has been assumed since 1992 that the cardioprotective (antiplatelet) effects of red wine are a result of its relatively high concentrations of polyphenolic compounds, specifically resveratrol (**1**).[4,24] In fact, since 1992 almost 900 research articles have appeared on this compound alone (PubMed search "resveratrol"). We were interested in the effects of resveratrol on the AA cascade, specifically on the COX portion of this pathway, since it has been well established that this portion of the cascade is involved in vascular homeostasis and it had been previously shown that COX was a putative target for resveratrol.[2,3,10−13] We reported that resveratrol specifically targets the COX-1 peroxidase active site and simultaneously inactivates the cyclooxygenase and peroxidase activities of the enzyme through a "hit-and-run" mechanism-based event (Scheme 2). Irreversible inactivation of COX-1 by resveratrol had an absolute requirement for the *m*-hydroquinone moiety of the drug and a peroxide substrate.[20]

The development of selective COX-1 inactivators has received limited attention since it is accepted that COX-2 is the desired target for NSAIDs.[25] However, aspirin is an effective cardioprotective agent that targets platelet-specific COX-1. Although it is not selective for this isoform, extremely high efficacy as an antiplatelet agent results from its ability to irreversibly inactivate COX. Irreversible inhibition can be surmounted only by new protein synthesis. Since platelets are unable to synthesize new protein, the effect of aspirin is governed by the $t_{1/2}$ of the platelet, which is 7 days. Thus a single low dose of aspirin can eliminate platelet TxA_2 synthesis for an extended period, while PGI_2 synthesis in the vascular endothelial cells can recover quickly. In this manner, aspirin shifts the TxA_2−PGI_2 balance to favor cardioprotection over thrombosis.[19]

Our hypothesis was that resveratrol (**1**) may have a pharmacology similar to aspirin with respect to its ability to inactivate platelet-specific COX-1. However, its unfavorable pharmacokinetics coupled with our findings, which indicate that its efficacy may be limited by the co-reductant and peroxide tone in the resting platelet, led us to consider other cardioprotective agents in red wine.[5−7,20] In the present study we showed that other *m*-hydroquinones found in red wine, namely, the catechins (**2**, **3**) and epicatechins (**4**, **5**), also act as mechanism-based inactivators of COX-1. These *m*-hydroquinones are as potent as resveratrol and are typically 15 times more concentrated then resveratrol in red wine.[21,22] These findings identify all dietary *m*-hydroquinones as potential mechanism-based inactivators of COX-1 and suggest that resveratrol may not be the sole agent responsible for the cardioprotective (antiplatelet) property of red wine.

Conclusions. The total *m*-hydroquinone content of red wine may be sufficient to mediate cardioprotection via the COX-1 pathway. First, effective plasma concentrations should be achievable for mixtures of *m*-hydroquinones, which when considered together are present at high micromolar concentrations in red wine.[21,22] Second, it is likely that daily consumption of red wine yields physiologically relevant concentrations of dietary *m*-hydroquinones in the plasma. Finally, only a small amount of the catechins (**2**, **3**) and epicatechins (**4**, **5**) need to be oxidized to cause COX-1 inactivation; therefore, it is likely that these dietary *m*-hydroquinones can surmount the low peroxide tone present in the resting platelet and yield significant COX-1 inactivation and lasting cardioprotection.

Experimental Section

Materials. Heme (FePPIX), AA, H_2O_2 (30% v/v), catechins, and epicatechins were purchased from Sigma. *N*,*N*,*N*′,*N*′-Tetramethyl-1,4-phenylenediamine (TMPD) was purchased from Arcos Organics. Resveratrol was purchased from Caymen Chemical. *m*-Dihydroxybenzene (resorcinol) was purchased from Aldrich.

Enzymes. COX-1 and human COX-2 were purified to homogeneity from ram seminal vesicles and baculovirus-infected *Sf*-21 insect cells, respectively.[26,27] The purified enzymes were obtained predominantly in their apo forms (>85%) and were reconstituted with at least 1 equivalent of heme cofactor (FePPIX) in the assay system prior to reaction initiation.

Peroxidase Activity Assay. The two-electron reduction of peroxide, using TMPD as the reducing co-substrate, was measured spectrophotometrically. The cuvette (1.0 mL) contained 100 mM Tris-HCl (pH 8.0), 2 μM FePPIX, 80 μM TMPD,

and 300 μM H_2O_2. The assays were initiated by the addition of peroxide. The formation of *N*,*N*,*N*′,*N*′-tetramethyl-1,4-phenylenediimine (E_{610} = 12 000 M^{-1} cm^{-1}) was complete within 60 s. By using this procedure, our COX-1 had a specific activity of 25 μmol of TMPD oxidized min^{-1} mg^{-1}.

Cyclooxygenase Activity Assay. The bis-dioxygenation of AA to yield PGG_2 was followed by measuring oxygen consumption using a Clark-style oxygen microelectrode (Instech). The standard assay chamber (600 μL) contained 100 mM Tris-HCl (pH 8.0), 1 mM phenol, 2 μM FePPIX, and 150 μM AA. The assays were initiated by the addition of AA. By using this procedure our COX-1 had a specific activity of 25 μmol of O_2 consumed min^{-1} mg^{-1}.

***K*$_d$ Measurements.** Difference spectroscopy was used to characterize the formation of an enzyme·drug complex. The cuvettes (1 mL) contained 100 mM Tris-HCl (pH 8.0), 5 μM FePPIX, and 2.5 μM enzyme. K_d values were determined by adding resveratrol or its analogues (10−500 μM) incrementally while monitoring complex formation at 404 nm with respect to an untreated sample. In this portion of the spectrum, all compounds were UV/vis transparent. Hyperbolic plots of Δabsorbance at 404 nm versus resveratrol (or analogue) concentration were obtained. Best estimates of K_d were obtained by iterative fits to the following equation for a hyperbola (the fits gave a mean ± standard deviation):

$$\Delta\text{absorbance} = (\Delta\text{absorbance}_{max} \times [I])/(K_d + [I])$$

RP-HPLC Analysis of COX-1-Dependent *m*-Hydroquinone Oxidation. Catalytic quantities of holo-COX-1 (0.2 units) were mixed with 50 μM analogue in 100 mM Tris-HCl (pH 8.0). The 1 mL reactions were initiated with the addition of 300 μM H_2O_2 and quenched after 2 min by the addition of 250 μL of 1 M sodium citrate (pH 4.0). Samples (100 μL) were injected onto a Waters Xterra RP_{18} column (3.5 μm; 4.6 × 150 mm) equilibrated with solvent A (10% methanol in water) at a flow rate of 0.75 mL/min. Beginning at 10 min, a linear gradient was run to solvent B (80% methanol in water) over 40 min, and isocratic flow was maintained at solvent B for an additional 10 min. This method was capable of separating analogues **1**−**6** (Figure 3). The column was returned to its initial conditions and equilibrated for 10 min prior to the next injection. Absorbance of the eluant was measured at 280 nm. The percent enzymatic oxidation of resveratrol and its analogues was calculated from RP-HPLC peak areas obtained in the presence and absence of H_2O_2 via the following equation:

$$\%\ \text{analogue oxidized} = (1 - (\text{peak area with } H_2O_2/\text{peak area without } H_2O_2)) \times 100.$$

The RP-HPLC analysis was performed using a Waters model 2695 pump equipped with a model 996 photodiode array detector.

Peroxidase-Dependent Inactivation of COX by *m*-Hydroquinones. Stoichiometric quantities of COX-1 or COX-2 (10 μM) were preincubated with mixtures of 100 μM H_2O_2 and 100 μM analogue in 100 mM Tris-HCl (pH 8.0) supplemented with 10 μM FePPIX for 5 min at 25 °C. The complete system contained all ingredients, while other systems lacked one or more ingredients. Preincubations were initiated with H_2O_2 (or H_2O when peroxide was not a reagent). Immediately following preincubation, the samples were diluted 200-fold into the peroxidase assay. Activity measurements were corrected for resveratrol carryover according to IC_{50} curves, and the percent activity remaining was computed with respect to an enzyme control.

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Note Added after ASAP: Errors in the structures in Scheme 1 appeared in the version posted on October 9, 2004. The corrected structures appear in the version posted on October 13, 2004.

References and Notes

(1) Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M.*Clin. Chim. Acta* **1995**, *235*, 207−219.
(2) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. *Science* **1997**, *275*, 218−220.
(3) Johnson, J. L.; Maddipati, K. R. *Prostaglandins Other Lipid Mediators* **1998**, *56*, 131−143.
(4) Renaud, S.; De Lorgeril, M. *Lancet* **1992**, *339*, 1523−1526.
(5) Bertelli, A.; Bertelli, A. A. E.; Gozzini, A.; Giovannini, L. *Drugs Exp. Clin. Res.* **1998**, *24*, 133−138.
(6) Aseni, M.; Ignacio, M.; Ortega, A.; Carretero, J.; Carmen Bano, M.; Obrador, E.; Estrela, J. M. *Free Radical Biol. Med.* **2002**, *33*, 387−398.
(7) Soleas, G. J.; Yan, J.; Goldbert, D. M. *J. Chromatogr. B* **2001**, *757*, 161−172.
(8) Samuelsson, B. *J. Am. Chem. Soc.* **1965**, *87*, 3011−3013.
(9) Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 899−903.
(10) Hamberg, M.; Svensson, J.; Wakabayashi, T.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 345−349.
(11) Hamberg, M.; Svensson, J.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2994−2998.
(12) Bunting, S.; Gryglewski, R.; Moncada, S.; Vane, J. R. *Prostaglandins* **1976**, *12*, 897−913.
(13) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature* **1976**, *263*, 663−665.
(14) Korbut, R.; Moncada, S. *Thrombosis Res.* **1978**, *13*, 489−500.
(15) Bunting, S.; Moncada, S.; Vane, J. R. *Br. Med. Bull.* **1983**, *39*, 271−276.
(16) Vane, J. R. *Nature* **1971**, *231*, 232−235.
(17) Amezcua, J. L.; O'Grady, J.; Salmon, J. A.; Moncada, S. *Thrombosis Res.* **1979**, *16*, 69−79.
(18) Patrono, C. *N. Engl. J. Med.* **1994**, *330*, 1287−1294.
(19) Patrono, C.; Coller, B.; Dalen, J. E.; FitzGerald, G. A.; Fuster, V.; Gent, M.; Hirsh, J.; Roth, G. *Chest* **2001**, *119* (Suppl. 1), 39S−63S.
(20) Szewczuk, L. M.; Forti, L.; Stivala, L. A.; Penning, T. M. *J. Biol. Chem.* **2004**, *279*, 22727−22737.
(21) Youyuan, P.; Chu, Q.; Liu, F.; Ye, J. *J. Agric. Food Chem.* **2004**, *52*, 153−156.
(22) Landrault, N.; Poucheret, P.; Ravel, P.; Gasc, F.; Cros, G.; Teissedre, P. L. *J. Agric. Food Chem.* **2001**, *49*, 3341−3348.
(23) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245−3249.
(24) Siemann, E. H.; Creasy, L. L. *Am. J. Enol. Vitic.* **1992**, *43*, 49−52.
(25) Hla, T.; Neilson, K. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7384−7388.
(26) Marnett, L. J.; Siedlik, P. H.; Ochs, R. C.; Pagels, W. R.; Das, M.; Honn, K. V.; Warnock, R. H.; Tainer, B. E.; Eling T. E. *Mol. Pharmacol.* **1984**, *26*, 328−335.
(27) George, H. J.; Marchand, P.; Murphy, K.; Wiswall, B. H.; Dowling, R.; Giannaras, J.; Hollis, G. F.; Trzaskos, J. M.; Copeland, R. A. *Protein Expression Purif.* **1996**, *7*, 19−26.

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