Abstract. Background/Aim: The flavonoid quercetin exerts significant anti-inflammatory activity against chronic infections, including periodontal disease. However, it is unclear whether combination of quercetin with other flavonoids enhances antioxidant and anti-inflammatory activity. To clarify the molecular mechanism responsible for the anti-inflammatory activity of quercetin, we investigated the antioxidant, cytotoxicity and anti-inflammatory activity of quercetin and its related compounds, catechin and epicatechin, and their combinations. Materials and Methods: Radical-scavenging activities were determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and cytotoxicity against RAW264.7 cells was determined using a cell counting kit (CCK-8). The inhibitory effects of these compounds on the mRNA expression of cyclooxygenase-2 (Cox2), tumor necrosis factor-alpha (Tnfα) and nitric oxide synthase 2 (Nos2), in RAW264.7 cells stimulated with Porphyromonas gingivalis (Pg) fimbriae, was also determined using real-time polymerase chain reaction analysis. The phenolic O-H bond dissociation enthalpy (BDE) and quantum chemical parameters were calculated on the basis of density function theory (DFT) BLYP/6-31G*. Results: The DPPH radical-scavenging activity (EC_{50}) of quercetin, catechin and epicatechin was 5.5, 7.7 and 6.2 μM, respectively, whereas the cytotoxicity (LC_{50}) was 4.45, 4.80 and 4.95 mM, respectively. Quercetin had slightly higher cytotoxicity and anti-DPPH activity than catechin and epicatechin. The BDE for the three flavonoids at the 4’-OH in the B ring, which is the initial active site, was about 75 kcal/mol. Furthermore, various combinations of quercetin with catechin or epicatechin exerted an antagonistic effect on anti-DPPH activity. Gene expression of Cox2, Tnfα and Nos2 stimulated by exposure to Pg-fimbriae was markedly suppressed by quercetin, but was not modulated by its combination with epicatechin. The 50% inhibitory concentration of quercetin for Cox2 expression was approximately 10 μM, while that of catechin and epicatechin was approximately 500 μM. Values of the quantum chemical parameters softness (σ) and electronegativity (χ) were highest for quercetin among the three flavonoids tested. Conclusion: The potent anti-inflammatory activity of quercetin appears to be attributable to its high σ and χ values. Quercetin may be applicable as a preventive agent against inflammatory periodontal disease as a manifestation of systemic disease.

Conditions such as diabetes mellitus (1), cardiovascular disease (2) and preterm low birth weight (3) are associated with an increased risk of chronic infections such as periodontitis. Porphyromonas gingivalis (Pg) is a key bacterium strongly associated with early-onset, progressive and refractory periodontal disease and subsequent alveolar bone loss. Pg-fimbriae, in addition to lipopolysaccharide (LPS), are also involved in the pathogenesis of periodontal disease through production of various pro-inflammatory cytokines, which promote the differentiation of osteoclasts (4-6). Pg-fimbriae are peritrichous filamentous appendages,
whereas LPS is a major component of the outer membrane of Pg and is capable of host activation, mediating the adhesion of bacteria to both host cells and a variety of oral substrates and molecules (7).

The polyphenols quercetin, catechin and epicatechin (Figure 1) are major flavonoids widely found in natural plants, and have become a focus of interest because of their various beneficial effects, including strong antioxidant, anti-inflammatory, and anticancer activities (8). In recent years, many reports have indicated that quercetin in particular exerts significant anti-microbial effects on periodontal pathogens in vitro (9). Quercetin is capable of interacting with a variety of enzymes including cyclooxygenase (COX), lipooxygenase, phosphodiesterase and tyrosine kinase, and is able to mediate anti-inflammatory processes, mainly by inhibiting the expression of COX1 and -2. Tumor necrosis factor-alpha (TNFα), an essential component of the immune system, is produced by several type cells, especially macrophages. Curcumin and various polyphenols have been shown to exert anti-inflammatory activity by binding directly to pro-inflammatory molecules such as COX1, -2 and TNFα. The antioxidant activity of various combinations of catechin with other polyphenols was previously investigated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and this revealed that some combinations exerted opposite effects, suggesting antagonism (10). However, the antioxidant and anti-inflammatory activity of various combinations of quercetin with catechin or epicatechin at the cellular level has not been sufficiently investigated. It would be of interest to clarify whether such combinations could positively or negatively modulate antioxidant and anti-inflammatory activity in cells because various mixtures of flavonoids are present in natural products.

In the present study, we investigated the inhibitory effects of quercetin, catechin and epicatechin and their combinations on the expression of Cox2, Tnfα and nitric oxide synthase 2 (Nos2) mRNAs stimulated by Pg-fimbriae in a murine macrophage cell line, RAW264.7. We also investigated the anti-DPPH activity of various combinations of quercetin with catechin or epicatechin in order to clarify their radical-scavenging activity resulting from phenolic interactions. Furthermore, in order to clarify the mechanism responsible for the biological activity of quercetin and its related compounds, the relationships between their cytotoxicity and anti-inflammatory activities were investigated in terms of quantum chemical parameters such as σ, χ and Koopman’s ionization potential (IP) (11).

**Materials and Methods**

**Materials.** (+)-Catechin and quercetin were purchased from Tokyo Kasei Co. (Tokyo, Japan). (−)-Epicatechin was obtained from Sigma Aldrich Japan Co. (Tokyo, Japan). The chemical structures of these compounds are shown in Figure 1. Solutions of these compounds were prepared by dissolving each of them in dimethyl sulfoxide, followed by dilution to the indicated concentrations using serum-free RPMI-1640 (Invitrogen Co., Carlsbad, CA, USA) as test samples. Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT, USA), and DPPH from Tokyo Kasei Co. (Tokyo, Japan).

**Anti-DPPH activity.** The radical-scavenging activities were determined using 0.1 mM DPPH as a free radical. In brief, each of the phenolic compounds was used at different concentrations in ethanol. The decrease in absorbance was determined at 540 nm for 30 min at room temperature. Radical absorbance activity was determined from the dose–response curves as the amount of inhibitor necessary to reduce the initial DPPH radical concentration. The 50% effective concentration (EC_{50}) for each compound was determined using the dose-response curves. Data are expressed as means of three independent experiments. Statistical analyses were performed using Student’s t-test.

**Phenolic interaction.** To evaluate possible interactions, various combinations of two phenolics were studied using the DPPH assay. The procedure employed was similar to that reported previously (10).

**Cell culture.** The murine macrophage-like cell line RAW264.7, obtained from Dainippon Sumitomo Pharma Biomedical Co. Ltd. (Osaka, Japan), was used. The cells were cultured to a subconfluent state in RPMI-1640 medium supplemented with 10% FBS at 37˚C and 5% CO₂ in air, washed, and then incubated overnight in serum-free RPMI-1640. They were then washed again and treated with the test samples.

**Cytotoxicity.** The relative number of viable cells was determined using a Cell Counting Kit-8 (CCK-8) (Dojindo Co., Kumamoto, Japan) (12). In brief, RAW264.7 cells (3×10⁴ per well) were cultured in NUNC 96-well plates (flat-well-type microculture plates) for 48 hours, after which the cells were incubated with test samples for 24 hours. CCK-8 solution was added to each well and then the absorbance was measured at 450 nm with a microplate reader (Biochromatic, Helsinki, Finland). The 50% lethal cytotoxic concentration (LC_{50}) was determined from the dose–response curves. Data are expressed as means of three independent experiments. Statistical analyses were performed using Student’s t-test.

**Preparation of Pg fimbriae.** P. gingivalis ATCC33277 fimbriae were prepared and purified from cell washings by the method of Yoshimura et al. (4). As documented previously, biological activities of purified fimbria-induced were not attributable to LPS contamination of the preparation (5, 6). Viability of the cells after exposure to the fimbriae at the concentrations used was over 90%, as determined using the Cell Counting Kit-8 (Dojindo Co.) (12). The protein content of the fimbriae was measured by the method of Smith et al. (13).

**Preparation of total RNA and real-time polymerase chain reaction (PCR).** The preparation of total RNA and the procedure for real-time PCR have been described previously (14). In brief, RAW264.7 cells in NUNC 96-flat-well-type microculture plates (10⁵ cells per
well) were treated with the test samples. Total RNA was isolated using an RNeasy Plus Micro Kit (Qiagen Japan Co. Ltd., Tokyo, Japan), in accordance with the instruction manual. cDNA was synthesized from total RNA (2 μg) of each sample by random priming using a High Capacity RNA-to-cDNA Kit (Life Technologies Japan, Tokyo, Japan). Reaction mixtures without the reverse transcriptase were also used as a negative control. An aliquot of each cDNA synthesis reaction mixture was diluted and used for real-time PCR quantification. An equal-volume aliquot of each cDNA was mixed, serially diluted, and used as a standard. TaqMan probes/primers for Cox2, Tnfα, Nos2, and 18S rRNA and the PCR enzyme mix for real-time PCR were purchased from Life Technologies Japan. Real-time PCR quantification was performed in triplicate using the GeneAmp Sequence Detection System 5700 software (Life Technologies Japan) in accordance with the instruction manual. The relative amount of target was calculated from standard curves generated in each PCR, and quantitative data with a coefficient of variance (CV) of less than 10% were used for further analyses. Each calculated amount of mRNA was standardized by reference to that for 18S rRNA. Data are expressed as means of three independent experiments. Statistical analyses were performed using Student’s t-test.

Computation. The phenolic O-H bond dissociation enthalpy (BDE) for the quercetin-related compounds was calculated as follows: Firstly, the lowest and second-lowest energy conformers of both the phenol derivatives and their phenoxyl radical species were identified as candidates for geometry optimization using the conformer search procedure by Merck Molecular Mechanics force fields calculation. The tentative conformers were then geometrically optimized by ab initio molecular orbital calculation at the HF/6-31G* level for the phenols, and with a UHF/6-31G* basis set for the phenoxyl radicals in vacuo, to afford the respective energetic minimized structures. The electronic energy was further preceded by single-point calculation involving density functional theory (DFT) using the B3LYP functional at the 6-31G* level: BDE=Hr + Hh − Hp, where

Figure 1. Chemical structures of catechin, epicatechin and quercetin and their initial oxidation products.
Hr is the enthalpy of the phenoxyl radical generated by hydrogen atom abstraction, Hh is the enthalpy of the hydrogen radical, and Hp is the enthalpy of the parent phenol. The lowest unoccupied molecular orbital (LUMO) energy (ELUMO) and highest occupied molecular orbital (HOMO) energy (E_HOMO) were obtained from the ground state equilibrium geometries with the calculated DFT B3LYP 6-31G* in vacuo from 6-31G* initial geometry (14, 15). The absolute value of HOMO energy was adopted as the IP value according to Koopman’s theorem (11). All calculations were performed using Spartan’10 (Wave Function Inc., Irvine, CA, USA). The chemical hardness (η), softness (σ) and electronegativity (χ) values were calculated as follows:

\[ \eta = \frac{(E_{\text{LUMO}} - E_{\text{HOMO}})}{2} \]  \hspace{1cm} (Eq. 1)
\[ \sigma = \frac{1}{\eta} \]  \hspace{1cm} (Eq. 2)
\[ \chi = -\frac{(E_{\text{LUMO}} + E_{\text{HOMO}})}{2} \]  \hspace{1cm} (Eq. 3)

Relationships between cytotoxicity or anti-inflammatory activity and quantum chemical parameters. IBM SPSS version 19 was used for performing correlations.

Results and Discussion

Antioxidant study. The anti-inflammatory activities of flavonoids are largely related to their ability to scavenge oxidative radicals, attenuate nuclear factor kappa B (NFkB) activity, inhibit several genes such as COX2, TNFA and NOS2 that are important for regulation of cells, and to prevent viral infection. Therefore, it is important to study the radical-scavenging activity of flavonoids. There have been many previous studies of in vitro radical-scavenging activity (10, 16-18). Firstly, using the DPPH assay, we investigated the effects of catechin, epicatechin and quercetin and their combinations, and the results are shown in Figure 2. The EC50 values for catechin, epicatechin and quercetin were 7.7, 6.2, and 5.5 μM, respectively. The radical-scavenging activity of quercetin was slightly higher than that of catechin and of epicatechin, with almost equivalent activity against DPPH radicals, being similar to results reported previously (10). To evaluate the antioxidant interaction of these flavonoids, we then examined their combinations using the DPPH assay. As shown in Table I, the calculated absorbance (A), the simple sum of the absorbance of both quercetin and catechin or epicatechin at 2 μM, was significantly smaller than the experimentally determined value (B), i.e. a mixture of quercetin with catechin or epicatechin to a total combined concentration of 4 μM. The absorbance ratio of A to B was 1.2-1.3, indicating that the anti-DPPH activity for A was greater than that for B. As a result, the combination of quercetin with catechin or epicatechin had an opposite effect on antioxidant activity, indicating an antagonistic effect. The radical-scavenging activity of quercetin in combination with catechin or epicatechin was balanced due to their
phenolic interaction during oxidation. Lacopini et al. reported that the scavenging properties of phenolic combinations of two, three or more catechin-related compounds using DPPH assay were antagonistic (10). On the other hand, in general, the radical-scavenging activity of phenolic compounds is dependent on the radical species. Some kinetic studies of radical-scavenging activity have been performed on quercetin and related compounds using tocopheroxy- and benzoyl peroxide (BPO)-radical species. Changes in the absorbance of the tocopheroxy radical at 20 nm during reaction of tocopheroxyl radicals with epicatechin or quercetin revealed that the second-order rate constant for epicatechin and quercetin was $1.52 \times 10^2$ M$^{-1}$ s$^{-1}$ and $2.98 \times 10^2$ M$^{-1}$ s$^{-1}$, respectively (17). The tocopheroxy radical-scavenging activity of quercetin is approximately twice that of epicatechin. In our previous study of radical-scavenging activity using the induction period method for polymerization of methyl methacrylate initiated by thermal decomposition of BPO, we found that the inhibition rate constant ($k_{inh}$) for catechin and quercetin was $1.05 \times 10^3$ M$^{-1}$ s$^{-1}$ and $1.60 \times 10^3$ M$^{-1}$ s$^{-1}$, respectively, the $k_{inh}$ value for quercetin being about 1.6 times higher than that for catechin (18). The radical-scavenging activity of combinations of quercetin and epicatechin using the induction period method also indicated an antagonistic effect (16). Our findings using the DPPH* assay were consistent with those reported above. Combinations of quercetin with catechin or epicatechin were concluded to negatively affect the antioxidant activity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A</th>
<th>B</th>
<th>A-B</th>
<th>B/A</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>15.0±0.7</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Epicatechin</td>
<td>15.1±0.2</td>
<td></td>
<td></td>
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<tr>
<td>Catechin</td>
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<tr>
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<td>30.1±0.9</td>
<td>35.0±0.8</td>
<td>4.9±0.1</td>
<td>1.16*</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Quercetin+catechin</td>
<td>21.4±0.9</td>
<td>27.0±0.3</td>
<td>5.6±0.6</td>
<td>1.26*</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Catechin+epicatechin</td>
<td>21.5±0.4</td>
<td>28.0±0.5</td>
<td>6.5±0.1</td>
<td>1.3*</td>
<td>Antagonism</td>
</tr>
</tbody>
</table>

The reduction rate was expressed as $|A_{540} \text{ of 0.1 mM DPPH} - A_{540} \text{ of sample}| / A_{540} \text{ of 0.1 mM DPPH} \times 100$. Values are the mean±SD (n=4). A: Calculated absorbance (%), simple sum of quercetin+epicatechin (or catechin), or catechin and epicatechin; B: experimentally recorded absorbance (%) for the indicated phenols and their mixtures. A vs. B, *p<0.01.
Cox2/Tnfa/Nos2 studies. Peroxynitrites and hydroxy radicals are derived from lipid peroxidation, disruption of cellular structures, inactivation of enzymes and ion channels via protein oxidation and nitration, and DNA damage (19). All these actions are associated with the onset and persistence of inflammation. Radical scavengers such as quercetin may prevent inflammation derived from LPS and bacterial fimbriae. Firstly, therefore, we investigated the cytotoxicity (LC50) of three flavonoids against RAW264.7 cells. The LC50 values for catechin, epicatechin and quercetin were 4,800, 4,950 and 4,450 μM, respectively (Figure 3). The cytotoxicity of quercetin was slightly higher than that of catechin and of epicatechin, but the difference in cytotoxicity between the compounds was very small.

Next, using RAW264.7 cells stimulated with Pg-fimbriae at non-cytotoxic concentrations, we investigated the expression of Cox2 and Tnfa mRNA after treatment with quercetin, catechin and epicatechin and their combinations. The results are also shown in Figures 4, and 5, respectively. For expression of Cox2 mRNA, the 50% effective concentration (Cox2IC50) of quercetin was approximately 10 μM, whereas that for both catechin and epicatechin was approximately 500 μM (Figure 6b). The anti-inflammatory activity of quercetin was approximately one-order of magnitude greater than that of catechin or epicatechin. It was considered that the anti-inflammatory activity of quercetin was potent, whereas that of catechin and epicatechin, steric isomers, were considerably weak. As shown in Figures 4b and 5b, the inhibitory activity of quercetin, catechin and epicatechin on Tnfa expression was almost the same as that on Cox2 expression. The inhibitory effect of the quercetin/epicatechin combination on Cox2 or Tnfa mRNA expression was also investigated, and results are shown in Figures 5a and b, respectively. The inhibitory effect of quercetin on both Cox2 and Tnfa expression was not enhanced by the addition of epicatechin. We incubated different concentrations of quercetin in the presence of 100 μM epicatechin and investigated its anti-inflammatory activity at molar ratios of 1, 2, 4 and 8. We found that the anti-inflammatory activity predominantly induced by quercetin and was not modulated by the addition of epicatechin. Addition of epicatechin to quercetin negatively affected the anti-inflammatory activity. This may be related to the antagonistic effect of the combination between quercetin and epicatechin on the radical-scavenging activity (Table I). The combination of quercetin with epicatechin was considered to have an opposite effect on antioxidant and anti-inflammatory activity.
Mu et al. reported that quercetin pre-treatment significantly inhibited NO production in LPS-stimulated RAW264.7 cells, the expression of Nos2 being markedly down-regulated via inhibition of Nf-κB (20). Therefore, we further investigated the inhibitory effects of catechin, epicatechin, quercetin and their combinations on Pg-fimbria-stimulated expression of Nos2 and Cox2 mRNA in RAW264.7 cells using real-time PCR. Figure 6 shows that quercetin at concentrations of both 25 μM and 50 μM strongly inhibited the fimbria-induced expression of Nos2, whereas catechin and epicatechin showed slight inhibition even at a relatively high concentration of 500 μM. Nos2 gene expression for combinations of quercetin and catechin or epicatechin at 50 μM was also similar to that for quercetin alone at the same concentration. The inhibitory effect of combinations of quercetin with catechin or epicatechin on Cox2 mRNA expression was also similar to that on Nos2 mRNA expression (Figure 6b). These findings appear to be consistent with the antagonistic effect of the radical scavenging activity of phenolic combinations determined using the anti-DPPH assay (Table I). The Nos2 mRNA expression after treatment with flavonoids was closely related to their antioxidant activity.

To date, most researchers have focused on the antioxidant and anti-inflammatory activity of quercetin-related compounds, however the mechanism and structural requirements for their antioxidant activity are not fully understood at the cellular level. One hypothesis to explain the inhibitory effect of quercetin on Pg-fimbria-stimulated expression of Nos2 and Cox2 mRNAs in RAW264.7 cells has stressed the importance of a catechol-like structure (the presence of 3’ and 4’ hydroxy groups in the B ring) and the C2-C3 double bond in the C ring (Figure 1) (21). However, catechin and epicatechin lack a C2-C3 double bond in the C ring. The inhibitory effect of quercetin on expression of Nos2 mRNA may be attributable to the presence of the C2-C3 double bond in the C ring. It was previously reported that the position, number and substitution of the hydroxy group in the B ring, and saturation of the C2-C3 double bond in the C ring. It was previously reported that the position, number and substitution of the hydroxy group in the B ring, and saturation of the C2-C3 double bond and polar groups on the C ring (22). We previously investigated the anti-inflammatory activity of curcumin and tetrahydrocurcumin and found that the activity of the former was markedly higher than that of the latter.
Table II. Cytotoxicity (50% cytotoxic concentration, LC50) and the ability to induce a 50% decrease in Porphyromonas gingivalis-fimbria-stimulated cyclo-oxygenase-2 (Cox2) mRNA expression (Cox2IC50) in RAW264.7 cells for flavonoids, and their quantum chemical parameters.

<table>
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<tr>
<th></th>
<th>Quercetin</th>
<th>Catechin</th>
<th>Epicatechin</th>
<th>Resveratrol</th>
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<td>LC50 (μM)</td>
<td>4.45</td>
<td>4.80</td>
<td>4.95</td>
<td>0.45</td>
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<td>EC50 (μM)</td>
<td>10</td>
<td>500</td>
<td>500</td>
<td>&lt;50, ca 30</td>
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<tr>
<td></td>
<td>5.481</td>
<td>5.664</td>
<td>5.683</td>
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<td></td>
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<td>(−5.63)</td>
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<td></td>
<td>−1.843</td>
<td>(−0.38)</td>
<td>0.01</td>
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<td></td>
<td>(−2.30)</td>
<td>(−0.38)</td>
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<td></td>
<td>5.481</td>
<td>5.664</td>
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<td>3.662</td>
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<td>2.836</td>
<td>3.23</td>
</tr>
</tbody>
</table>

*Ref. (27), †Ref. (24), ‡Ref. (25). Note: The lowest unoccupied molecular orbital (LUMO) energy (E_{LUMO}), highest occupied molecular orbital (HOMO) energy (E_{HOMO}), ionization potential (IP)=|E_{HOMO}|, η=(E_{LUMO}−E_{HOMO})/2, σ=1/η, χ=−(E_{LUMO} + E_{HOMO})/2, calculations at DFT-B3LYP/6-31G* level.

possibly due to the fact that curcumin has phenol rings connected by two α, β-unsaturated carbonyl groups (double bond), whereas tetrahydrocurcumin lacks the unsaturated carbonyl group (23). Interestingly, phenolic interactions between quercetin and epicatechin or catechin negatively affected the anti-inflammatory activity and therefore the synergistic anti-inflammatory activities of quercetin in combination with other flavonoids are questionable. However, a more detailed study is necessary to substantiate this hypothesis.

**BDE, IP, σ and χ.** Quantum chemical calculation might provide a clearer insight into the molecular mechanisms of radical-scavenging and anti-inflammatory activity of these phenolic compounds. The HOMO and LUMO energy for three flavonoids and resveratrol were calculated, and the results are shown in Table II. The HOMO and LUMO values for quercetin, catechin and epicatechin were similar to those reported previously (24, 25). Since BDE and IP for flavonoid antioxidants can represent radical-scavenging to a large extent, we calculated the BDE and IP values for flavonoids at the DFT-B3LYP/6-31G* level. The BDE values for flavonoids (see Figure 1) are shown in Table III. The BDE of 4'-OH (kcal/mol), the initial active site in the B-ring for flavonoids, declined in the order catechin (75.9) > epicatechin (75.3) > quercetin (75.1). By contrast, the corresponding BDE for resveratrol at 3'-OH (the initial active site in the B-ring) was 80.77 kcal/mol, being about 5 kcal/mol higher than that for flavonoids. The IP value is the most important energy factor for evaluation of scavenging ability (11). A relatively high IP value decreases the rate of electron transfer between a phenolic antioxidant and oxygen, and therefore phenolic compounds with a higher IP value have lower pro-oxidative potency, and *vice versa* (26). The IP value (eV) declined in the order catechin ≈ epicatechin (5.7) > quercetin (5.4) > resveratrol (5.3) (Table II). It has been shown experimentally that the anti-DPPH* activity of quercetin, catechin or epicatechin with a catechol-like substitution on the B ring is about 8 times greater than that of resveratrol with a mono phenol on the B ring (EC50 45 μM) (27). In general, low BDE and IP values indicate a high antioxidant activity, but an extremely low IP value may result in a change from an antioxidant to a prooxidant character. The low anti-DPPH* activity of resveratrol may be related to its high BDE value. The cytotoxicity of resveratrol was also one order of magnitude greater than that of quercetin, catechin or epicatechin (Table II), possibly due to the small IP value of this compound. Phenolic compounds with a small IP value tend to act as pro-oxidants in the presence of reactive oxygen species and increase the cytotoxic potential of phenolics.

Molecules with a relatively small HOMO-LUMO energy gap (E_{HOMO} − E_{LUMO}) are generally reactive, while those with a relatively large value are generally not reactive (15). As shown in Table II, the softness (σ) value (σ=1/η; i.e. the inverse value of hardness) of quercetin was highest among the three flavonoids tested, indicating that quercetin is more reactive than catechin or epicatechin. In contrast, the χ value represents the chemical potential of the electrophile. According to the Frontier Orbital Theory, adduct formation occurs when a soft nucleophile donates its highest-energy electrons to the empty lowest-energy orbital of a soft electrophile. Hence, the most relevant frontier orbital for electrophiles is the LUMO, whereas the HOMO is most important for nucleophiles. The σ value is defined as the ease with which electron re-distribution can take place during covalent bonding, and thus the softer the electrophile (i.e. the more negative the E_{LUMO} value and the higher the σ value), the more readily it will form an adduct by accepting outer-shell electrons from a soft nucleophile such as a sulfur atom (1). Investigation of the relationship between anti-inflammatory activity and quantum chemical parameters for
resveratrol-related compounds has recently revealed that resveratrol, that has potent anti-inflammatory activity, has a large χ value, but also a small η value, *i.e.* a large σ value (27). Here we investigated the correlation between cytotoxicity or anti-inflammatory activity, in terms of inhibition of Cox2 mRNA expression, and the σ, IP, and χ values for four polyphenols: quercetin, catechin, epicatechin, and resveratrol (Table II). The results are shown in Table IV. There were no significant relationships of σ, IP or χ with cytotoxicity. By contrast, the logarithmic value of anti-inflammatory activity (Cox2IC50) for these polyphenols had a significant linear relationship to the σ and to the χ values, suggesting that Cox2IC50 may be dependent on σ or χ. These data clearly demonstrate that the potent inhibitory effect of quercetin on Pg-fimbria-induced Cox2 expression in RAW264.7 cells may be related to its high σ or χ value, although some other mechanism may also contribute to the net effect.

It has been considered that polyphenols, capable of interacting with and modulating the activity of a variety of enzymes including COX, lipoxygenase, phosphodiesterase and tyrosine kinase, are the most potent non-steroidal anti-inflammatory drug-like compounds. It has been proposed that quercetin has an inhibitory effect on protein tyrosine kinase (28) and inhibits the interaction of carcinogens with DNA (29). Quercetin supplementation has also been reported to up-regulate the mRNA and protein levels of the intercellular antioxidant enzyme paraoxonase 2 (Pon2) in cultured RAW264.7 cells (30). The beneficial effects of quercetin in biological systems may be associated with its interaction with enzymes, as it possesses antioxidant activities that prevent free radical damage to biological molecules. Quercetin has high σ and χ values, allowing it to bind to the active site cavity with a favorable ligand–protein molecular interaction. On the other hand, oxidized flavonoids readily and specifically adduct thiol groups, and may disrupt vital cellular compounds containing such groups (31). When quercetin is incubated in the presence of glutathione, a quinoid derivative is produced through hydroperoxidase action (32). Quinoid derivatives exert adverse effects in biological systems. Since quercetin possesses high σ and χ values, it may exert stronger pro-inflammatory activity and toxic effects in the presence of ROS. A quinone derived through the oxidation of quercetin would preferentially attack macromolecules (DNA and enzymes) in biological systems due to the reactivity of unsaturated compounds with nucleophilic additions (33, 34). It is likely that quercetin can scavenge many intercellular free radicals, and the various metabolites of quercetin, including quercetin-derived radicals, are probably involved in toxicity. More detailed studies of the anti-inflammatory and antioxidant actions of quercetin and related compounds will be required in order to understand their true potential as preventive agents against chronic infections and various oral diseases, including cancer.

### Acknowledgements

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