Antioxidant and Cyclooxygenase-2-inhibiting Activity of 4,4’-Biphenol, 2,2’-Biphenol and Phenol

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Abstract. The anthropogenic substance 4,4’-biphenol and its analogues are estrogenic and cytotoxic. It has been previously found that synthesized ortho-dimers of phenolic compounds possess potent antioxidative and anti-inflammatory activity. To clarify the relationships between radical-scavenging and anti-inflammatory activities, the radical-scavenging activities of 4,4’-biphenol, 2,2’-biphenol and phenol were investigated by using differential scanning calorimetry to measure the induction period for polymerization of methyl methacrylate initiated by thermal decomposition of 2,2’-azobisisobutyronitrile. We also investigated the inhibitory effects of these compounds on lipopolysaccharide (LPS)-stimulated cyclooxygenase-2 (COX-2) mRNA and protein expression and on binding of activator-protein-1 (AP-1) and nuclear factor kappa-B (NF-kB) to their respective consensus sequences. Cytotoxicity declined in the order 4,4’-biphenol > 2,2’-biphenol >> phenol. 2,2’-Biphenol, but not 4,4’-biphenol, showed inhibitory effects on LPS-stimulated COX-2 expression and on AP-1 and NF-kB binding to their consensus sequences at 1-10 μM. Expression of COX-2 in RAW cells was enhanced by 4,4’-biphenol plus LPS, possibly because of radical-mediated transformation of 4,4’-biphenol to the cytotoxic diphenylquinone, as judged by the stoichiometric factor (n value) of 3.429 and low IP Koopman value of this biphenol. In contrast, the anti-inflammatory activity of 2,2’-biphenol may be the result of the formation of a dimer derived from oxidation of this compound, as suggested by its n value close to 1. Phenol showed anti-inflammatory activity but did not completely inhibit COX-2 expression, even at higher concentrations. Synthetic phenols such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are well-known antioxidants. We have previously synthesized various phenolic dimers have been previously synthesized and their antioxidant activity investigated their antioxidant activity as well as their cytotoxicity and ability to inhibit cyclooxygenase-2 (COX-2) in RAW264.7 cells (1). It was found that some dimers derived from ortho-ortho coupling of the parent monomers possess potent antioxidant and anti-inflammatory activity (2-7). To evaluate the antioxidant activity of a series of phenolic dimers, we have previously used differential scanning calorimetry (DSC) has been used to measure the induction period for the polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of 2,2’-azobisisobutyronitrile (AIBN) or benzoyl peroxide (BPO) under nearly anaerobic conditions (3). This method has proved to be reliable for evaluating the activity of these compounds (2). The anthropogenic chemicals bisphenol-A and 4,4’-biphenol are well known to possess very high estrogenic activity (8), but bisphenol-A and its related derivatives were previously reported to have no significant effect on cyclooxygenase-1 and -2 activities at concentrations up to 100 μM (9). It has been previously found that ortho-biphenols such as eugenol, dimer and 2-methoxy-4-methyl-phenol dimer as well as BHA dimer possess potent antioxidative activity and COX-2 inhibitory activity (2-7). Thus, it is of interest to establish whether para- or ortho-biphenols possess higher antioxidative and anti-inflammatory activity.
This study investigated the antioxidant and anti-inflammatory activity of 4,4'-'biphenol, 2,2'-biphenol and phenol. To do so, we assessed their radical-scavenging activities by determining the induction period for polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of AIBN. An investigation was also performed on, we determined whether these phenolic compounds could inhibit LPS-stimulated COX-2 mRNA expression as well as binding of activator protein-1 (AP-1) and nuclear factor kappa-B (NF-κB) to their respective consensus sequences in RAW264.7 cells. Furthermore, we calculated theoretical parameters were calculated for the biphenols, such as bond dissociation enthalpy (BDE) and ionization potential (IP) in accordance with Koopman's theorem (IP_koopman) (10) at the density functional theory (DFT)/B3LYP levels. The biological activities of 4,4'-biphenol, 2,2'-biphenol and phenol are discussed in the light of physicochemical parameters such as stoechiometric factor (n), BDE and IP_koopman.

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: 2,2'-biphenol, 4,4'-biphenol, phenol, and methyl methacrylate (MMA) (Tokyo Kasei Kogyo, Co., Ltd., Tokyo, Japan). The chemical structures of the phenols used are shown in Figure 1. AIBN (Wako Pure Chemical Industries Ltd., Japan) was re-crystallized from chloroform. Megaprime DNA labeling system, 5'-[α-32P]dCTP, and [γ-32P]ATP were purchased from Amersham Biosciences Co. (Piscataway, NJ, USA). A 5'-end labeling system was purchased from Promega Co. (Madison, WI, USA). A mouse COX-2 cDNA probe with a length of approximately 1.2 kbp was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). A β-actin oligonucleotide probe was purchased from GeneDetect.com Ltd. (Bradenton, FL, USA). COX-2 goat polyclonal antibody, β-actin rabbit polyclonal antibody, and HRP-conjugated mouse anti-goat IgG were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). HRP-conjugated goat anti-rabbit IgG and Phototope-HRP Western blot detection kit came from Cell Signaling Technology, Inc. (Beverly, MA, USA). RPMI-1640 was purchased from Invitrogen Corp. (Carlsbad, CA, USA). Fetal bovine serum (FBS) was from HyClone (Logan, UT, USA). *Escherichia coli* O111 B4-derived LPS was from List Biological Laboratories, Inc. (Campbell, CA, USA).

Cell culture. Cells of the murine macrophage-like cell line RAW 264.7, obtained from the Dainippon Sumitomo Pharma Biomedical Co. Ltd. (Osaka, Japan), were used. They 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 treated with or without LPS (100 ng/ml), and their total RNA was prepared 3 h later by the acid guanidine phenol chloroform (AGPC) procedure (13). The RNA was electrophoresed in 1% agarose gels with 0.2 M sodium phosphate as a running buffer and blotted onto nylon membranes (Micron Separations, Inc., Westboro, MA, USA). The membranes were then hybridized with COX-2 cDNA probe labeled with 5'-[alpha-32P]dCTP by the Megaprime DNA labeling system (Amersham Biosciences Co.) and β-actin oligonucleotide probe labeled with [γ-32P]ATP by a 5'-end labeling system purchased from Promega Co. After hybridization, the membranes were washed and dried, then exposed overnight to Kodak X-ray film (Eastman Kodak Co., Rochester, NY, USA) at -70˚C. β-Actin was used as an internal standard for quantification of total RNA in each lane of the gel. Quantification of COX-2 expression was carried out by densitometry. The data are expressed as the relative signal intensity (percentage of maximum).

Northern blot analysis. The procedure was similar to that previously reported (12). Briefly, 106 cells were placed in Falcon 5 cm-diameter dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) and pretreated for 30 min with the indicated dose of phenols. They were then treated with or without LPS (100 ng/ml), and then the absorbance was measured at 450 nm with a microplate reader (Biochromatic, Helsinki, Finland). The 50% cytotoxic concentration (CC50) was determined from the dose-response curves. Data were expressed as means of three independent experiments. Statistical analyses were performed by Student's t-test.

Western blot analysis. Cells in Falcon 5-cm-diameter dishes (106 cells per dish) were treated with test samples. Then the cells were solubilized with lysis buffer (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% [vol/vol] Triton X-100, 2.5 mM sodium pyrophosphate) 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% cytotoxic concentration (CC50) was determined from the dose-response curves. Data were expressed as means of three independent experiments. Statistical analyses were performed by Student’s t-test.
pyrophosphate, 2 mM Na2VO4, 10 mM NaF, 1 mM β-glycerolphosphate, 1 μg/ml aprotinin, 1 mM PMSF). Protein concentrations were measured by the method of Smith et al. (14). Each sample (10 μg of protein) was subjected to SDS-PAGE in a 12.5% polyacrylamide gel, and the separated proteins were transferred to a polyvinylidene difluoride membrane (Millipore Co., Bedford, MA, USA). Then, the blots were blocked with 5% skim milk, washed, and incubated with anti-COX-2 antibody as primary antibody diluted 1:1000 in working solution (5% BSA, 1 x TBS [50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl], and 0.1% Tween 20) at 4°C. β-Actin antibody was used at 0.1 μg/ml after dilution with working solution. After incubation, the blots were treated at room temperature with HRP-conjugated secondary antibody diluted 1:4000. Proteins were detected with a Phototope-HRP Western blot detection kit (Cell Signaling Technology, Inc.), and the blots were exposed to Kodak x-ray film for 10 min. β-Actin was used as a loading control in each lane of the gel.

Preparation of nuclear extract and electro mobility shift assay (EMSA). Nuclei were extracted and prepared for the gel mobility shift assay as reported previously (12). In brief, the cells in Falcon 15 cm diameter dishes (10⁶ cells per dish) were pretreated for 30 min with or without the indicated dose of phenols and then were treated with LPS at 100 ng/ml for 1 h. Thereafter, the cells were scraped into phosphate-buffered saline, pelleted and suspended in lysis buffer containing 10 mM Tris-HCl (pH 7.4), 3 mM MgCl2, 10 mM NaCl, and 0.5% Nonidet P-40. The nuclei were separated from the cytosol by centrifugation at 3,000 × g for 15 min. The extracted nuclei were then treated with buffer A (10 mM HEPES [pH 7.9], 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol [DTT]) and further treated by stirring for 60 min at 4°C in buffer B (20 mM HEPES [pH 7.9], 1.5 mM MgCl2, 0.2 mM EDTA, 0.42 M NaCl, 25% glycerol, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride (PMSF)). Nuclear extracts were obtained by centrifugation for 60 min at 25,000 × g and demineralized through a Sephadex G-25 column equilibrated with buffer C (20 mM HEPES [pH 7.9], 0.1 M KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF). Protein concentrations were measured by the method previously reported (14). Binding reactions were performed for 20 min at room temperature with 10 μg of the nuclear proteins in 2 mM Tris (pH 7.5) containing 8 mM NaCl, 0.2 mM EDTA, 0.8% (v/v) glycerol, 0.2 mM DTT, 0.5 mM PMSF, 1 μg of poly (dl-dc) and 20,000 cpm of 32P-labeled NF-κB or AP-1 oligonucleotides in a final volume of 20 μl. Poly (dl-dc) and nuclear extract were incubated at 4°C for 10 min before the polymerization of MMA. The induction period (IP) and the initial rates of polymerization in the absence (Rpcon) and presence (Rpinh) of a phenolic inhibitor were calculated by a previously reported procedure (3).

Measurement of stoichiometric factor (n). The relative n value can be calculated from the induction period in the presence of inhibitors as follows:

\[
\text{n} = \frac{R_i[\text{IP}]}{[\text{IH}]}
\]

where [IP] is the induction period in the presence of an inhibitor [IH]. The number of moles of peroxy or alkyl radicals trapped by the antioxidant was calculated with respect to 1 mole of inhibitor moiety unit. The RI value for AIBN at 70°C was 5.56×10⁻⁶ mol l⁻¹ s⁻¹ (3).

The antioxidant activity of phenolic compounds can be evaluated using stoichiometric factor, n values. The higher the n value, the higher the antioxidant activity. In general, as a maximum value, the n value of phenolic compounds with two OH groups such as 4,4'-biphenol and 2,2'-biphenol shows 4, whereas that of phenol with one OH group shows 2. When the n value of phenolic compounds shows about 1, dimer could be produced due to the radical-radical coupling reaction derived from oxidized phenolic monomers.

Computation. The phenolic O-H bond dissociation enthalpy (BDE) was calculated as follows. First, the lowest energy and second-lowest energy conformers of both the phenol derivatives and their phenoxyl radical species were identified as candidates for geometry optimization by using the conformer search procedure of the MMFF (Merck molecular mechanics) force fields calculation. Then, the tentative conformers were optimized in geometry by ab initio molecular orbital calculations on a Hartree-Fock model with ab initio 6-31G* (HF/6-31G*) for the phenols and UHF/6-31G* level for the phenoxyl radicals in vacuo to afford the respective energetic minimized structures. The electronic energy calculation further proceeded by single point calculation of density functional theory (DFT) using the B3LYP functional on the 6-31G* basis set (5, 6). Then, BDE = Hr + Hh – Hp, where Hr is the enthalpy of the phenoxyl radical generated by H-abstraction, Hh is the enthalpy of the hydrogen radical, and Hp is the enthalpy of the parent phenol. The energy values of both the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the fully optimized phenol derivatives were calculated by the HF/6-31G* level basis set molecular orbital procedure. The absolute value of HOMO energy was adopted as an approximate ionization potential value (IPkoopman) according to Koopman’s theory (10). All of the molecular modeling and calculation were performed with Spartan 04 (Wavefunction Inc., Irvine, CA, USA).

Results
Induction period and propagation rate. The results are shown in Figure 2 and Table I. Linear relationships were observed between induction period or Rpinh/Rpcon and
concentration for 4,4'-biphenol, 2,2'-biphenol and phenol. The induction period declined in the order 4,4'-biphenol > 2,2'-biphenol > phenol. The slope of $R_{pinh}/R_{pcon}$ declined in the order 4,4'-biphenol > phenol > 2,2'-biphenol. 

Values of $n$ and $R_{pinh}/R_{pcon}$ are shown in Table I. The $n$ values of 4,4'-biphenol, 2,2'-biphenol and phenol were 3.29, 1.33 and 0.90, respectively. In contrast, $R_{pinh}/R_{pcon}$ was 0.94, 0.78 and 0.66 for 4,4'-biphenol, phenol and 2,2'-biphenol, respectively. 4,4'-Biphenol gave an $n$ value of 3.29, suggesting the formation of diphenylquinone during the induction period. The $n$ value of this compound when fully oxidized is 4. Phenol and 2,2'-biphenol showed $n$ values of about 1, suggesting the formation of dimers after one-electron oxidation and/or H-atom abstraction, possibly due to recombination of the corresponding aryloxy radicals. The $R_{pinh}/R_{pcon}$ value of about 1 for 4,4'-biphenol suggested lower reactivity with growing MMA radicals (macromolecule polymer radicals) than that of 2,2'-biphenol or phenol, suggesting that the reaction products of oxidized 4,4'-biphenol, diphenylquinones, are not able to suppress free radicals derived from unsaturated lipid peroxidation. In contrast, the reaction products of oxidized phenol and 2,2'-biphenol, particularly the latter, would be able to suppress such free radicals.

**BDE and IP.** The results are shown in Table II. The BDE and IP values of 4,4'-biphenol were considerably different from those of 2,2'-biphenol. BDE$_{1st}$ for 2,2'-biphenol was much lower than that of 4,4'-biphenol, whereas BDE$_{2nd}$ of 4,4'-biphenol was lower than that of 2,2'-biphenol. In contrast, the IP$_{1st}$ and IP$_{2nd}$ of 4,4'-biphenol were lower than those of 2,2'-biphenol and phenol. The induction period declined in the order 4,4'-biphenol > 2,2'-biphenol > phenol. The slope of $R_{pinh}/R_{pcon}$ declined in the order 4,4'-biphenol > phenol > 2,2'-biphenol.

**Table I.** Radical-scavenging activity of biphenols and their cytotoxicity toward RAW 264.7 cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$n^a$</th>
<th>$R_{pinh}/R_{pcon}^b$</th>
<th>CC$_{50}$ mM$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,4'-Biphenol</td>
<td>3.29</td>
<td>0.94</td>
<td>0.25</td>
</tr>
<tr>
<td>2,2'-Biphenol</td>
<td>1.33</td>
<td>0.66</td>
<td>0.55</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.9</td>
<td>0.78</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*a*Stoichiometric factor (the number of radicals trapped by each inhibitor molecule), *b*the ratio of the initial rate of polymerization with an inhibitor ($R_{pinh}$) and that without an inhibitor ($R_{pcon}$), the mean of three different experiments (errors <5% ). *c*The mean of 50% cytotoxic concentration for three different experiments (errors <15%). 4,4'-Biphenol vs. 2,2' Biphenol, $p<0.01$. 2,2'-Biphenol or 4,4'-Biphenol vs. Phenol, $p<0.001$.

**Table II.** Phenolic O-H bond dissociation enthalpy (BDE) and ionization potential (IP$_{Koopman}$) for biphenols.

<table>
<thead>
<tr>
<th>Compound</th>
<th>BDE$_{1st}$ (kJ/mol)</th>
<th>BDE$_{2nd}$ (kJ/mol)</th>
<th>IP (eV)</th>
<th>IP$_{2nd}$ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,4'-Biphenol</td>
<td>346.14</td>
<td>379.61</td>
<td>5.348</td>
<td>5.444</td>
</tr>
<tr>
<td>2,2'-Biphenol</td>
<td>287.35</td>
<td>449.1</td>
<td>5.97</td>
<td>5.665</td>
</tr>
<tr>
<td>Phenol</td>
<td>359.5</td>
<td></td>
<td>5.957</td>
<td></td>
</tr>
</tbody>
</table>

Values were determined using DFT/B3LYP/6-31G$^*$. 

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Figure 2. Plots of the induction period (left panel) or the ratio of the rate of propagation with a phenol inhibitor to that without an inhibitor (control), $R_{pinh}/R_{pcon}$ (right panel) vs concentrations of phenol inhibitors. The procedures are described in Materials and Methods.
2.2'-biphenol. Phenol showed the largest BDE and IP, indicating its lower antioxidant activity compared with the biphenols. The AIBN radical is a strong electron/H-atom abstracting agent and is expected to react rapidly with biphenols to form quinones or other compounds. 2,2'-Biphenol may be preferentially dimerized because its BDE₁st is markedly smaller than that of 4,4'-biphenol, whereas the BDE₂nd of 2,2'-biphenol is much higher than that of 4,4'-biphenol, so that 2,2'-biphenol preferentially undergoes one-electron oxidation. On the other hand, 4,4'-biphenol with its low IP value may well enhance pro-oxidant activity under conditions of oxidative stress.

Figure 3. Regulatory effects of 4,4'-biphenol on LPS-stimulated expression of the COX-2 gene in RAW 264.7 cells. Left panel: The procedure is described in Materials and Methods. Blotting analysis was performed to determine COX-2 mRNA expression level. Right panel: Bar graph of level of COX-2 mRNA. The results are presented as means ± standard error (SE), which was <15%. The statistical significance of the differences between the untreated control and the treated groups (0.1, 1 and 10 μM) were determined, and showed a p value of <0.01 for 1 and 10 μM. Three independent experiments were performed, and similar results were obtained.

Figure 4. Regulatory effects of 2,2'-biphenol and phenol on LPS-stimulated expression of the COX-2 gene in RAW 264.7 cells. Left panel: The procedure is described in Materials and Methods. Blotting analysis was performed to determine COX-2 mRNA expression level. Right panel: Bar graph of level of COX-2 mRNA. The results are presented as means ± standard error (SE), which was <15%. Statistical significance of the differences between the LPS-only group and the treated groups (each 1, 10 and 100 μM): p<0.01 for 1 μM 2,2'-biphenol, p<0.05 for 10 μM 2,2'-biphenol, p<0.1 for 1 μM phenol. Three independent experiments were performed, and similar results were obtained.
Cytotoxicity. The results are shown in Table I. Cytotoxicity declined in the order 4,4’-biphenol > 2,2’-biphenol > > phenol.

Induction of COX-2 expression by biphenols. To examine the effect of biphenols on LPS-stimulated COX-2 expression in RAW 264.7 cells, the cells were exposed to 0.1–100 μM of biphenols for 3 h. The results are shown in Figures 3 and 4, respectively. 4,4’-Biphenol up-regulated COX-2 mRNA expression at low concentrations (0.1–10 μM; Figure 3), whereas up-regulation by 2,2’-biphenol required a higher concentration (100 μM; Figure 4). Relative signal intensity (% of maximum) was significantly enhanced about 20–30% by treatment with 1–10 μM 4,4’-biphenol compared with the control (Figure 3). However, 4,4’-biphenol alone did not induce COX-2 mRNA expression (Figure 5). In addition, phenol caused no up-regulation of COX-2 mRNA expression at the concentrations tested.

COX-2 inhibition. Next, we investigated the inhibitory effects of 4,4’-biphenol, 2,2’-biphenol and phenol on LPS-stimulated COX-2 mRNA expression were investigated in RAW cells (Figure 4). 2,2’-Biphenol significantly inhibited COX-2 expression at a concentration of 1–10 μM, whereas phenol did not cause 50% inhibition of COX-2 expression under similar conditions. Western blotting was then used to assess the inhibitory effects of 2,2’-biphenol and phenol on LPS-stimulated COX-2 protein production in the same cells. Figure 6 shows that 2,2’-biphenol inhibited COX-2 production at concentrations of 1–10 μM, whereas phenol only weakly inhibited COX-2 production. In contrast, 4,4’-biphenol did not inhibit the LPS-stimulated COX-2 mRNA expression (data not shown).

Inhibition of AP-1 and NF-κB binding using EMSA. An EMSA was used to examine whether AP-1 binding to the 12-tetradecanoylphorbol-13-acetate-responsive element (TRE) sequence in LPS-stimulated RAW cells could be inhibited by 2,2’-biphenol, which is a COX-2 inhibitor. As shown in Figure 7, LPS stimulated AP-1 binding to the TRE and this binding was markedly inhibited by 2,2’-biphenol at 1–10 μM. In addition, LPS-stimulated binding of AP-1 was completely inhibited by an unlabelled oligonucleotide containing the TRE (data not shown). We also investigated whether 2,2’-biphenol could inhibit LPS-stimulated binding of NF-κB to its consensus sequence. As shown in Figure 8, 2,2’-biphenol at concentrations of 1–10 μM clearly inhibited NF-κB binding. These findings suggest that 2,2’-biphenol is a potent inhibitor of LPS-triggered cellular signaling.

Discussion

In general, radical-mediated phenolic toxicity is a multi-step process and is affected by the nature of the rate-determining step. Previously, it was shown that one or a combination of mechanisms, i.e. phenoxy radical or intermediates (quinone methides, quinones), contribute to phenol toxicity (15). The formation of intermediates probably affects the cytotoxic binding step to cellular membranes. The cytotoxicity of 4,4’-biphenol may be a result of formation of a quinone. It was previously reported that, following bioactivation using a peroxidase/H2O2 system, inhibitory effects on topoisomerase II were observed at concentrations as low as 50 μM for both phenol and 2,2’-biphenol, and 10 μM for 4,4’-biphenol. The addition of reduced glutathione (GSH) to the 4,4’-biphenol and horseradish peroxidase reaction system protected topoisomerase II from inhibition (16). The conversion of 4,4’-biphenol to its diphenylquinone may deplete antioxidants such as GSH. The arylating diphenolquinones derived from oxidized 4,4’-biphenol may react with cellular nucleophiles such as thiols on cysteine residues of proteins and glutathione (GSH), forming covalently linked quinone–thiol Michael adducts. When GSH is consumed, diphenolquinones can cause toxicity. The DNA-cleaving activity of 4,4’-biphenolic acid was has been previously reported, possibly due to the large O2 consumption and H2O2 generation activity of this compound (17). This activity may be responsible for the formation of quinone derived from 4,4’-biphenol because since quinones produce reactive oxygen species (ROS) through redox cycling with semiquinone radicals, directly or indirectly. In addition, quinones induce oxidative stress by the generation of ROS and reduction of cellular GSH (18). The formation of 4,4’-diphenolquinone from oxidized 4,4’-biphenol was indicated by its stoichiometric factor, n=3.429, in the present study. By contrast, n values of about 1 for 2,2’-biphenol and phenol suggest the formation of dimers.

The COX-2 gene encodes an inducible prostaglandin synthase enzyme that is overexpressed in tumors. COX-2 is not detected in most normal cells, but is rapidly induced by LPS, lipid peroxides, oxidant stress, cytokines (IFN-alpha, IL-1beta, IL-6), and TGF-beta 1 (19, 20). The murine COX-2 gene contains numerous cis-acting promoter elements, including NF-κB and AP-1 sites (21). Thus, the transcription of COX-2 is probably modulated by NF-κB and AP-1 in macrophages (21). 4,4’-Biphenol was a potent inducer of COX-2 mRNA expression at concentrations as low as 50 μM for both phenol and 2,2’-biphenol, and 10 μM for 4,4’-biphenol.
expression in RAW cells. This may be related to the formation of 1,4′-diphenylquinone via the enzymatic and/or non-enzymatic oxidation of 4,4′-biphenol. In contrast, although 2,2′-biphenol at the high concentration of 100 μM enhanced the expression of COX-2 mRNA, it also significantly inhibited LPS-stimulated COX-2 expression at low concentrations (1-10 μM). Phenol also inhibited COX-2 expression, but did not reach 50% inhibition at any concentration tested. Acenaphthe-
nequinone was has been previously reported to modulate COX-2 expression through ROS generation and NF-κB activation in A549 cells (22). This supports our the suggestion that induction of COX-2 expression by 4,4’-biphenol involves its conversion to diphenylquinone and consequently the generation of ROS and activation of NF-κB. The low IP value of 4,4'-biphenol may be responsible for its conversion to diphenylquinone, because compounds with a low IP value preferentially undergo ionization.

In contrast, 2,2'-biphenol possesses anti-inflammatory activity, as shown by the findings that binding of AP-1 and NF-κB to their respective consensus sequences in LPS-stimulated cells, and also LPS-stimulated COX-2 expression, were inhibited by 2,2'-biphenol. The formation of dimers or oligomers via oxiation may be responsible for the anti-inflammatory activity of 2,2'-biphenol. However, the mechanism by which biphenols inhibit COX-2 expression is still unclear. Further studies are needed to elucidate the mechanism of the anti-inflammatory effects of biphenols.

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References


