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Myricetin Down-Regulates Phorbol Ester-Induced Cyclooxygenase-2 Expression in Mouse Epidermal Cells by Blocking Activation of Nuclear Factor Kappa B

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Abnormal expression of cyclooxygenase-2 (COX-2) has been implicated in the development of cancer. There are multiple lines of evidence that red wine exerts chemopreventive effects, and 3,5,4′-trihydroxy-*trans*-stilbene (resveratrol), which is a non-flavonoid polyphenol found in red wine, has been reported to be a natural chemopreventive agent. However, other phytochemicals might contribute to the cancer-preventive activities of red wine, and the flavonol content of red wines is about 30 times higher than that of resveratrol. Here we report that 3,3′,4′,5,5′,7-hexahydroxyflavone (myricetin), one of the major flavonols in red wine, inhibits 12-*O*-tetradecanoylphorbol-13-acetate (phorbol ester)-induced COX-2 expression in JB6 P+ mouse epidermal (JB6 P+) cells by suppressing activation of nuclear factor kappa B (NF-κB). Myricetin at 10 and 20 μM inhibited phorbol ester-induced upregulation of COX-2 protein, while resveratrol at the same concentration did not exert significant effects. The phorbol ester-induced production of prostaglandin E_2 was also attenuated by myricetin treatment. Myricetin inhibited both COX-2 and NF-κB transactivation in phorbol ester-treated JB6 P+ cells, as determined using a luciferase assay. Myricetin blocked the phorbol ester-stimulated DNA binding activity of NF-κB, as determined using an electrophoretic mobility shift assay. Moreover, TPCK (*N*-tosyl-L-phenylalanine chloromethyl ketone), a NF-κB inhibitor, significantly attenuated COX-2 expression and NF-κB promoter activity in phorbol ester-treated JB6 P+ cells. In addition, red wine extract inhibited phorbol ester-induced COX-2 expression and NF-κB transactivation in JB6 P+ cells. Collectively, these data suggest that myricetin contributes to the chemopreventive effects of red wine through inhibition of COX-2 expression by blocking the activation of NF-κB.

KEYWORDS: Chemoprevention; Cyclooxygenase-2; Myricetin; nuclear factor kappa B; Tumor promotion

INTRODUCTION

There are multiple lines of evidence of an association between inflammatory tissue damage and the development of cancer (*1*). Recent research has shown that both human and experimental tumors contain increased amounts of prostaglandin E_2 (PGE_2), which modulates cytokine balance, inhibits host immunity, and may play an important role in carcinogenesis (*2*). Cyclooxygenase (COX), an important enzyme involved in mediating the inflammatory process, produces PGE_2 from endogenous arachidonic acid (*3*). Several isoforms of COX have been reported. COX-1, the constitutive isoenzyme that is expressed in most tissues, controls homeostasis by maintaining the physiological level of prostaglandins. Conversely, inducible COX-2 is dramatically up-regulated by a wide variety of stimuli such as cytokines, mitogens, oncogenes, growth factors, and tumor promoters and is detectable in only certain types of tissue (*1, 4*). Elevated levels of PGE_2 and enhanced COX-2 activity are frequently observed in a variety of malignancies, including those of the breast, prostate, bladder, liver, pancreas, skin, lung, colon, and brain (*5–7*). Conversely, COX-2 inhibitors, such as celecoxib, piroxicam, sulindac, and aspirin, have been shown to reduce the formation and growth of experimentally induced cancers in animals (*8–10*). Therefore, the suppression of prostaglandin synthesis through the selective inhibition of COX-2 is now regarded as a promising and practical approach to cancer prevention. The 5′-flanking region of the COX-2 promoter contains two putative binding sites for the eukaryotic transcription factor nuclear factor kappa B (NF-κB) (*11*). NF-κB has been shown to up-regulate COX-2 in diverse cell types,

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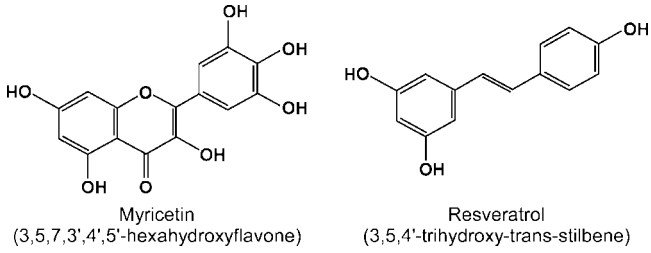

Figure 1. Chemical structures of myricetin and resveratrol.

with recent studies showing that NF-κB is a crucial component of the intracellular signaling pathways responsible for inducing COX-2 expression (*12*).

Chemoprevention refers to the use of drug or natural substances to prevent, retard, or reverse carcinogenesis and represents one of several promising strategies to inhibit the development of cancer. Recently, there has been considerable public and scientific interest in the use of phenolic substances derived from dietary components because of their potential chemopreventive activities. The anticancer activity of 3,5,4′-trihydroxy-*trans*-stilbene (resveratrol, **Figure 1**), a non-flavonoid polyphenol found in red wine, has been extensively studied and is well-established both *in vitro* and *in vivo* (*13, 14*). Resveratrol was found to inhibit lipopolysaccharide- and 12-*O*-tetradecanoylphorbol-13-acetate (phorbol ester)-induced COX-2 expression in BV-2 microglial cells (*15*) and human mammary epithelial cells (*16*), respectively. *In vivo*, the administration of resveratrol reduced COX-2 expression in an experimentally induced colitis model (*15*). Resveratrol inhibited the modulation of intracellular signaling pathways involved in aberrant COX-2 expression in phorbol ester-stimulated mouse skin (*13, 17*). From previous studies, the approximate concentration range at which resveratrol exerts potent protective effects against the development of tumors is 40–200 μM, while the achievable tissue concentration of resveratrol is in the low micromolar range (*18*). The resveratrol content of French red wines is 0.6–6.8 mg/L, and the tissue concentration required to exert chemoprevention effects may be difficult to achieve by drinking only one or two glasses of red wine a day (*18, 19*).

The flavonol concentration in red wine is about 30 times higher that of resveratrol. The major flavonol components in red wine are 3,3′,4′,5,5′,7-hexahydroxyflavone (myricetin, **Figure 1**) and 3,3′,4′,5,7-pentahydroxyflavone (quercetin) (*20*), which typically represent 20–50% of the total flavonol content (*20*). Flavonols occur widely in nature in plants, including tea, berries, and vegetables (*21*). Several studies have shown that myricetin exhibits anticarcinogenic activities. Studies have indicated that myricetin has a potent antioxidant capacity (*22*) and suppresses cancer development induced by polycyclic aromatic hydrocarbons in SENCAR mice (*23*). It has also been demonstrated that myricetin exerts protective effects against two-stage skin tumorigenesis (*24*) and inhibits the growth of A549 lung cancer cells (*25*). In colorectal cancer cells, myricetin inhibits the activity of matrix metalloproteinase-2 (*26*). Here we report that myricetin exerts stronger inhibitory effects than resveratrol on phorbol ester-induced upregulation of COX-2 expression in JB6 P+ mouse epidermal (JB6 P+) cells by targeting the NF-κB pathway.

MATERIALS AND METHODS

Chemicals. Myricetin, resveratrol, *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK), and phorbol ester were purchased from Sigma-Aldrich (St. Louis, MO). Eagle's minimum essential medium (MEM) was purchased from Mediatech (Herndon, VA). Fetal bovine serum (FBS) and penicillin/streptomycin were obtained from GIBCO BRL (Grand Island, NY). Rabbit polyclonal COX-2 antibody was obtained from Cayman Chemical (Ann Arbor, MI), and β-actin antibodies were obtained from Sigma-Aldrich. BCA protein assay reagents were obtained from Pierce (Rockford, IL), and the enhanced chemiluminescence (ECL) detection kit was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Luciferase assay substrate was purchased from Promega (Madison, WI), and the NF-κB DNA binding protein detection system and Trizol reagent were purchased from GIBCO BRL.

Preparation of Red Wine Extracts (RWE). Red wine (Merlot, California) was gently evaporated to remove alcohol at 50 °C under reduced pressure, and finally the concentrated residue was lyophilized and finely sprayed to obtain RWE dry powder (*27*).

Cell Culture. The JB6 P+ cell line obtained from the American type Culture Collection (Manassas, VA) was maintained in MEM supplemented with 5% (v/v) FBS and penicillin/streptomycin at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. This cell line was stably transfected with a COX-2 or NF-κB luciferase reporter plasmid that were generously provided by Dr. Chuanshu Huang (New York University) and Dr. Zigang Dong (University of Minnesota), respectively. These cells were cultured in monolayers at 37 °C in MEM containing 5% heat-inactivated FBS, 2 mM L-glutamine, and 25 μg/mL gentamicin in a humidified atmosphere containing 5% CO_2.

Western Blot Analysis. After the cells (1.5×10^6) were cultured in a 10 cm dish for 48 h, they were starved in serum-free medium for a further 12 h to eliminate the influence of FBS on the expression of COX-2. After treatment, cells were scraped and then treated with RIPA lysis buffer [150 mM NaCl, 0.5% Triton X-100, 50 mM Tris-HCl (pH 7.4), 25 mM NaF, 20 mM EGTA, 1 mM dithiothreitol (DTT), 1 mM Na_3VO_4, and a protease inhibitor cocktail tablet] for 40 min on ice followed by centrifugation at 14 800*g* for 30 min The protein concentration of the supernatant was measured using the BCA reagents. Protein (30 μg) was loaded on a 12% sodium dodecyl sulfate–polyacrylamide electrophoresis gel and electrotransferred onto a poly(vinylidene difluoride) membrane in Tris-glycine buffer (pH 8.4) containing 20% methanol. The membrane was then blocked in 5% fat-free dry milk in phosphate-buffered saline (PBS) with 0.1% Tween-20 for 1 h. The membranes were incubated with 1:5000-diluted β-actin (Sigma-Aldrich) or 1:1000-diluted COX-2 (Cayman Chemical) at room temperature. After three 5 min washes with PBS/0.1% Tween-20, the membranes were incubated with a 1:5000-diluted horseradish peroxidase-conjugated secondary antibody for 1 h. The transferred proteins were visualized with the ECL Western blot detection system according to the manufacturer's instructions (Amersham Pharmacia Biotech).

PGE_2 Assay. JB6 P+ cells (1.5×10^6) were plated in six-well dishes and grown to 80% confluence in 2 mL of growth medium. The cells were then treated with 10 ng/mL phorbol ester in the absence or presence of myricetin and resveratrol. The amounts of PGE_2 released into the medium were measured using the PGE_2enzyme immunoassay kit (Cayman Chemical).

Luciferase Assay. Confluent monolayers of JB6 P+ cells that were stably transfected with a COX-2 or NF-κB luciferase reporter plasmid were trypsinized, and 5×10^3 viable cells suspended in 100 μL of 5% FBS MEM were added to each well of a 96-well plate that was incubated at 37 °C in a

humidified atmosphere of 5% CO_2. When cells reached 80–90% confluence, they were starved by culturing them in 0.1% FBS MEM for a further 24 h. The cells were then treated for 1 h with myricetin (10 and 20 μM) and then exposed to 10 ng/mL phorbol ester for 24 h. After treatment, cells were disrupted with 100 μL of lysis buffer [0.1 M potassium phosphate buffer (pH 7.8), 1% Triton X-100, 1 mM DTT, and 2 mM EDTA], and the luciferase activity was measured using a luminometer (Luminoskan Ascent, Thermo Electron, Helsinki, Finland).

Electrophoretic Mobility Shift Assay (EMSA). An EMSA was performed using a DNA protein-binding detection kit (Gibco BRL, Grand Island, NY) according to the manufacturer's protocol. Briefly, the NF-κB oligonucleotide probe (50-AGT TGA GGG GAC TTT CCC AGG C-30) was labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase and purified on a Nick column (Amersham Pharmacia Biotech). The binding reaction was carried out in a total volume of 25 mL containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM DTT, 1 mM EDTA, 4% (v/v) glycerol, 0.1 mg/mL sonicated salmon sperm DNA, 10 mg of nuclear extracts, and the labeled probe at 100 000 cpm After 50 min of incubation at room temperature, 2 mL of 0.1% bromophenol blue was added, and samples were electrophoresed through a 6% nondenaturating polyacrylamide gel at 150 V in a cold room for 2 h. Finally, the gel was dried and exposed to X-ray film.

Statistical Analysis. Data are expressed as mean ± SD values. Student's *t* test was used for single statistical comparisons, with a probability value of $p < 0.05$ used as the criterion for statistical significance.

RESULTS

Myricetin Suppressed the Phorbol Ester-Induced COX-2 Expression in JB6 P+ Cells. We found that the expression of COX-2 in JB6 P+ cells was maximal after 6 h of treatment with 10 ng/mL phorbol ester (data not shown). To evaluate the inhibitory effects on the proinflammatory gene expression, cells were pretreated with or without myricetin or resveratrol at concentrations of 10 and 20 μM for 30 min. Compared with phorbol ester-induced COX-2 expression, treatment with 10 and 20 μM myricetin (but not with resveratrol) significantly inhibited phorbol ester-induced COX-2 expression (**Figure 2**). These results indicate that myricetin is more potent than resveratrol at inhibiting phorbol ester-induced COX-2 expression in JB6 P+ cells.

Myricetin Inhibited Phorbol Ester-Induced PGE_2 Production in JB6 P+ Cells. As a consequence of the increased COX-2 expression, the production of PGE_2 (which is one of the major products of COX-2) peaked at 16 h following treatment with phorbol ester (data not shown). Myricetin at 10 μM inhibited phorbol ester-induced PGE_2 production by 40%, while resveratrol at the same concentration did not exert significant effects (**Figure 3**). These results indicate that myricetin was substantially more effective than resveratrol in suppressing phorbol ester-induced PGE_2 production in JB6 P+ cells.

Myricetin Attenuated Phorbol Ester-Induced Activation of COX-2 Promoter Activity. To investigate whether the inhibition of COX-2 expression is attributable to transcriptional regulation, we determined the transcriptional activity of COX-2 using a luciferase assay. Phorbol ester increased COX-2 transcriptional activity in JB6 P+ cells, which was suppressed by 66% and 90% by myricetin at concentrations of 10 and 20 μM, respectively (**Figure 4A**). By contrast, resveratrol at concentrations of 10 and 20 μM inhibit phorbol ester-induced

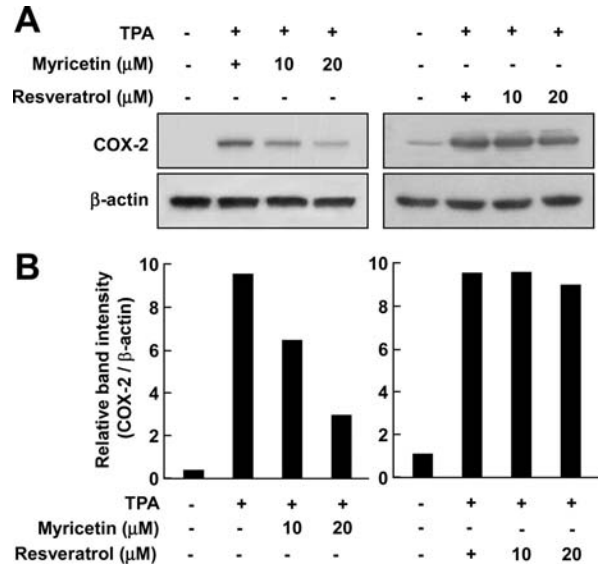

Figure 2. Effects of myricetin and resveratrol on phorbol ester-induced up-regulation of COX-2. JB6 P+ cells were pretreated with myricetin or resveratrol at the indicated concentrations (10 and 20 μM) for 30 min before incubation with 10 ng/mL phorbol ester for 4 h. (**A**) The levels of COX-2 and β-actin proteins were determined by Western blot analysis as described in the Materials and Methods section using specific antibodies. Data are representative of two independent experiments that gave similar results. (**B**) COX-2 expression was analyzed using the Image J program.

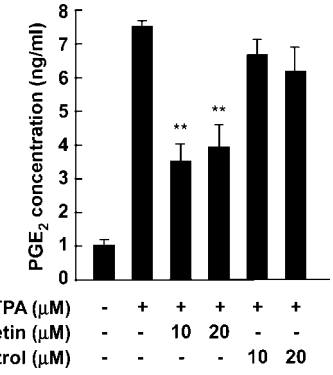

Figure 3. Effects of myricetin and resveratrol on phorbol ester-induced generation of PGE_2. JB6 P+ cells were pretreated with myricetin or resveratrol at the indicated concentrations (10 and 20 μM) for 30 min before incubation with 10 ng/mL phorbol ester for 16 h. PGE_2 generation was determined by the PGE_2 assay kit as described in the Materials and Methods section. The asterisk (*) indicates a significant difference between groups treated with phorbol ester and myricetin or resveratrol and the group treated with phorbol ester alone ($p < 0.05$).

COX-2 promoter activity by only 16% and 21%, respectively (**Figure 4A**). We further evaluated the potential synergistic effects of these two polyphenols on phorbol ester-induced COX-2 promoter activity. We treated cells with phorbol ester and myricetin (10 μM) combined with increasing doses of resveratrol (0–20 μM). However, results indicated that, instead of acting synergistically, the compounds seemed to have an additive inhibitory effect on COX-2 promoter activity (**Figure 4B**).

Myricetin Blocked Phorbol Ester-Induced NF-κB Transactivation. There are multiple lines of evidence that NF-κB is a key regulator of COX-2 expression in many types of cancer cells. To determine whether the inhibition of COX-2 expression by myricetin involves the inhibition of NF-κB activation, we

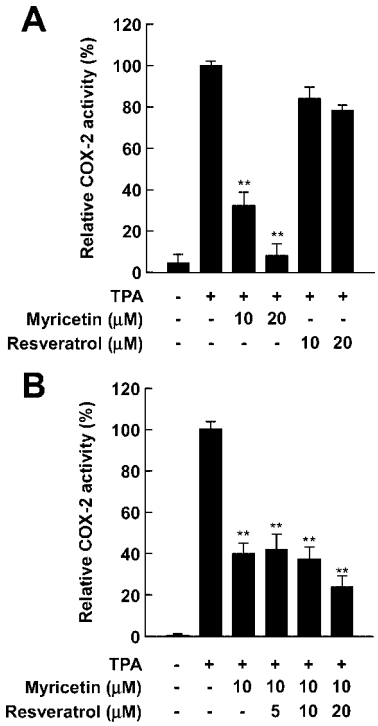

Figure 4. Effects of myricetin and resveratrol, alone and in combination, on phorbol ester-induced COX-2 promoter activity. For the luciferase assay, JB6 P+ cells that were stably transfected with a COX-2 luciferase reporter were cultured as described in the Materials and Methods section. (**A**) The cells were treated or not treated with myricetin or resveratrol at the indicated concentrations (10 and 20 μM) for 1 h before they were exposed to 10 ng/mL phorbol ester for 24 h. (**B**) The cells were treated or not treated with myricetin (10 μM) and resveratrol at the indicated concentrations (0, 5, 10, and 20 μM) for 1 h before they were exposed to 10 ng/mL phorbol ester for 24 h. COX-2 activity was measured using a luciferase assay and is expressed relative to control cells that were not treated with phorbol ester. Data represent mean ± SD values of the COX-2 luciferase activity calculated from three independent experiments. The asterisk (*) indicates a significant difference between groups treated with phorbol ester and myricetin or resveratrol, alone and in combination, and the group treated with phorbol ester alone ($p < 0.05$).

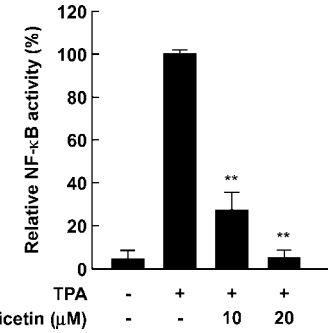

Figure 5. Effects of myricetin on phorbol ester-induced activation of NF-κB in JB6 P+ cells. For the luciferase assay, JB6 P+ cells that were stably transfected with an NF-κB luciferase reporter were cultured as described in the Materials and Methods section. The cells were treated or not treated with myricetin at the indicated concentrations (10 and 20 μM) for 1 h before they were exposed to 10 ng/mL phorbol ester for 24 h. NF-κB activity was measured using a luciferase assay and is expressed relative to control cells that were not treated with phorbol ester. Data represent mean ± SD values of the NF-κB luciferase activity calculated from three independent experiments. The asterisk (*) indicates a significant difference between groups treated with phorbol ester and myricetin and the group treated with phorbol ester alone ($p < 0.05$).

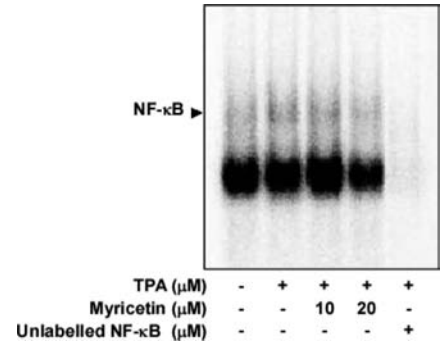

Figure 6. Effect of myricetin on phorbol ester-induced NF-κB DNA-binding activity. JB6 P+ cells were pretreated with myricetin (10 and 20 μM) for 30 min followed by incubation with 10 ng/mL phorbol ester for 1 h. Nuclear extracts were prepared and an EMSA was performed as described in the Materials and Methods section.

measured NF-κB transactivation using JB6 P+ cells that were stably transfected with an NF-κB luciferase reporter plasmid. Phorbol ester induced a significant increase in the luciferase activity, and this was blocked by myricetin at concentrations of 10 and 20 μM (**Figure 5**).

Myricetin Inhibited Phorbol Ester-Induced DNA Binding Activity of NF-κB in JB6 P+ Cells. We next examined the effects of myricetin on phorbol ester-stimulated DNA binding of NF-κB in JB6 P+ cells. Nuclear extracts obtained from phorbol ester-treated JB6 P+ cells, with or without myricetin pretreatment, were subjected to an EMSA using the oligonucleotide harboring the NF-κB-binding sequence present in the mouse COX-2 promoter region. Myricetin inhibited phorbol ester-induced DNA binding of NF-κB in JB6 P+ cells (**Figure 6**).

TPCK Inhibited the Phorbol Ester-Induced COX-2 Expression and NF-κB Transactivation. To demonstrate that NF-κB signaling is directly involved in the regulation of COX-2 expression in JB6 P+ cells, we investigated whether a specific NF-κB inhibitor (TPCK) could suppress the phorbol ester-induced expression of COX-2. Pretreatment of cells with TPCK at concentrations of 10 and 20 μM for 30 min significantly reduced phorbol ester-induced COX-2 expression (**Figure 7A**,**B**). We also found that the phorbol ester-induced NF-κB promoter activity was suppressed by TPCK treatment (**Figure 7C**).

RWE Attenuated the Phorbol Ester-Induced COX-2 Expression and NF-κB Transactivation. We next examined the effects of RWE on phorbol ester-induced COX-2 expression in JB6 P+ cells. Compared with phorbol ester-induced COX-2 expression, treatment with 10 or 20 μg/mL RWE significantly inhibited the phorbol ester-induced COX-2 expression in JB6 P+ cells (**Figure 8A**,**B**). RWE strongly inhibited phorbol ester-induced transactivation of NF-κB in JB6 P+ cells (**Figure 8C**). These results supported that the chemopreventive effects of red wine may be attributable mainly to flavonoids such as myricetin rather than resveratrol.

DISCUSSION

There are multiple lines of evidence that a dietary intake of red wine contributes to the "French paradox", which refers to the unexpectedly low cardiovascular morbidity in French people despite this population consuming a diet that is rich in saturated fats. It has been demonstrated that resveratrol may be relevant to the reported cardiovascular benefits of drinking wine.

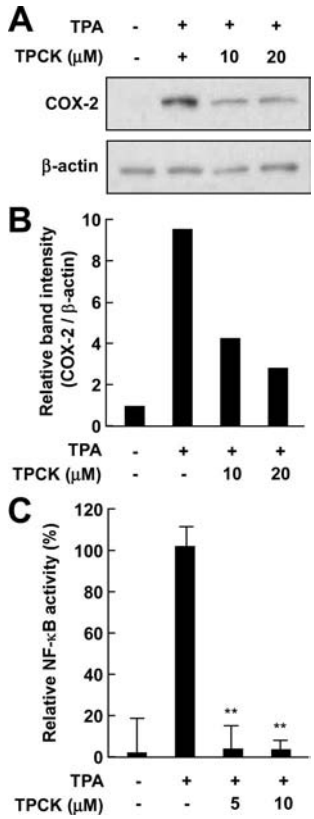

Figure 7. Effects of TPCK on the COX-2 expression and NF-κB activity induced by phorbol ester. (**A**) TPCK inhibited phorbol ester-induced COX-2 expression in JB6 P+ cells. JB6 P+ cells were pretreated with TPCK (10 and 20 μM) for 30 min before incubation with 10 ng/mL phorbol ester for 4 h. The levels of COX-2 and β-actin proteins were determined by Western blot analysis as described in the Materials and Methods section using specific antibodies against the corresponding phosphorylated and total proteins. Data are representative of two independent experiments that gave similar results. (**B**) COX-2 expression was analyzed using the Image J program. (**C**) TPCK suppressed phorbol ester-induced NF-κB transactivation. JB6P+ cells that were stably transfected with an NF-κB luciferase reporter were cultured as described in the Materials and Methods section. The cells were treated or not treated with TPCK at the indicated concentrations (5 and 10 μM) for 1 h before they were exposed to 10 ng/mL phorbol ester for 24 h. NF-κB activity was measured using a luciferase assay and is expressed relative to control cells that were not treated with phorbol ester. The asterisk (*) indicates a significant difference between groups treated with phorbol ester and TPCK and the group treated with phorbol ester alone ($p < 0.05$).

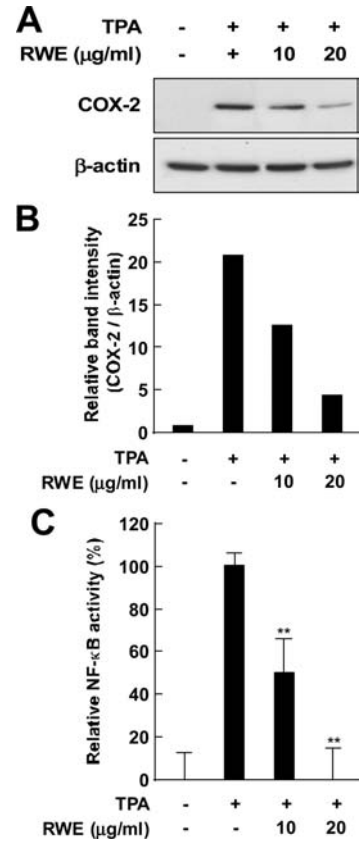

Figure 8. Effects of RWE on the COX-2 expression and NF-κB activity induced by phorbol ester. (**A**) RWE inhibited phorbol ester-induced COX-2 expression in JB6 P+ cells. JB6 P+ cells were pretreated with RWE (10 and 20 μg/mL) for 30 min before incubation with 10 ng/mL phorbol ester for 4 h. The levels of COX-2 and β-actin proteins were determined by Western blot analysis as described in the Materials and Methods section using specific antibodies against the corresponding phosphorylated and total proteins. Data are representative of two independent experiments that gave similar results. (**B**) COX-2 expression was analyzed using the Image J program. (**C**) RWE suppressed phorbol ester-induced NF-κB transactivation. JB6P+ cells that were stably transfected with an NF-κB luciferase reporter were cultured as described in the Materials and Methods section. The cells were treated or not treated with RWE at the indicated concentrations (10 and 20 μg/mL) for 1 h before they were exposed to 10 ng/mL phorbol ester for 24 h. NF-κB activity was measured using a luciferase assay and is expressed relative to control cells that were not treated with phorbol ester. The asterisk (*) indicates a significant difference between groups treated with phorbol ester and RWE and the group treated with phorbol ester alone ($p < 0.05$).

Recently, it has been shown that resveratrol can act as a potential cancer chemopreventive agent, and a great deal of experimental effort has been directed toward defining this effect. Resveratrol directly inhibited the activity of COX-2 and phorbol ester-induced carcinogenesis in a mouse skin cancer model (*13, 16*). However, despite the accumulating evidence of the chemopreventive potential of resveratrol, the content of flavonols such as myricetin and quercetin in red wines is much higher than that of resveratrol. In the present study, we found that myricetin at 10 and 20 μM exhibited strong inhibitory effects on the tumor-promoter-induced upregulation of COX-2 expression in JB6 P+ cells, while resveratrol at the same concentrations had no effect. Myricetin also effectively suppressed PGE_2 production in a non-concentration-dependent manner, while resveratrol again had no significant effect.

There is considerable evidence of the role of COX-2 and its products, especially PGE_2, in the development of cancer. The expression of COX-2 has been detected in various neoplasms, including breast, colon, and skin cancers (*28–30*). It has shown that skin tumors from premalignant actinic keratosis to squamous cell carcinoma overexpress COX-2 protein and that basal PGE_2 production is higher compared to normal skin (*30, 31*). Evidence that COX-2 is directly related to carcinogenesis comes from experiments showing that COX-2-knockout mice are resistant to experimental carcinogenesis (*32*). Abnormal overexpression of COX-2 blocks apoptosis and potentiates the invasiveness of malignant cells, which are reduced by nonsteroidal anti-inflammatory drugs such as celecoxib and sulindac (*11*). The specific COX-2 inhibitor celecoxib has shown chemopreventive effects in animal studies by potently reducing skin tumor formation and increasing tumor latency (*33, 34*). Thus, COX-2 has been recognized as a molecular target of many

chemopreventive and anti-inflammatory agents. We found that myricetin was substantially more potent than resveratrol at inhibiting COX-2 expression and that it has chemopreventive potential.

The NF-κB signaling pathway has been implicated in the expression of COX-2 stimulated by various stimuli (*35*, *36*). The 5′-flanking region of the COX-2 promoter contains binding sites for several transcription factors, including NF-κB (*37*), which reportedly regulates phorbol ester-induced COX-2 expression in mouse skin (*38*). Thus, putative anticarcinogenic activity may be due to the inhibition of phorbol ester-induced NF-κB activation. NF-κB is constitutively present in cells as a heterodimer, consisting of a p50 DNA binding subunit and a p65 transactivating subunit. NF-κB is normally held in the cytoplasm in an inactivated state by the inhibitor protein IκB-α. Exposure of cells or tissues to oxidative stimuli/tumor promoters results in free NF-κB translocating to the nucleus after dissociation of the cytosolic NF-κB/IκB-α complex. The present study found that myricetin strongly blocked the phorbol ester-induced transactivation and DNA binding activity of NF-κB in JB6 P+ cells. Furthermore, incubation with an NF-κB inhibitor, TPCK, suppressed the phorbol ester-induced COX-2 expression as well as NF-κB activation. These findings indicate that NF-κB plays a pivotal role in the COX-2 expression of JB6 P+ cells and is a critical molecular target for the chemopreventive effects of myricetin.

Our study also demonstrated that RWE strongly inhibited phorbol ester-induced COX-2 expression through blocking activation of NF-κB in JB6 P+ cells. Although the concentration of myricetin required to exert these effects may be difficult to be achieved by drinking only one or two glasses of red wine a day (*39*), other flavonoids such as oligomoeric procyanidins and anthocyanidin could contribute the observed cancer-preventive activities of red wine. In summary, myricetin inhibits phorbol ester-induced COX-2 expression in JB6 P+ cells by suppressing the activation of NF-κB. Our results suggest that myricetin has potent chemopreventive activity and might contribute to the chemopreventive potential of several foods, including red wine.

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