Evaluation of Food-derived Functional Ingredients According to Activation of PPAR and Suppression of COX-2 Expression

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The peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors belonging to the nuclear receptor family. They are considered molecular targets for the prevention of lifestyle-related diseases and are involved in the control of cyclooxygenase (COX)-2 expression. COX-2, the rate-limiting enzyme in prostaglandin biosynthesis, plays a key role in inflammation and circulatory homeostasis, and its expression is partly controlled by PPAR. We have identified several natural chemicals, such as resveratrol, that activate PPARs and suppress COX-2 expression. In this review, we provide an evaluation of food-derived functional ingredients that target PPARs and COX-2.

Keywords: PPAR, COX-2, resveratrol, essential oil, anti-lifestyle-related disease

Introduction

The prevention of lifestyle-related diseases, such as cardiovascular disease, diabetes, and stroke, is of worldwide interest. Individuals tend not only to focus on drug therapy for prevention of these diseases, but also on the functionality of natural chemicals found in food and beverages, such as polyphenols and their polymers. A growing body of evidence supports the theory that polyphenolic compounds have activities that maintain health. Epidemiological studies have demonstrated that dietary polyphenol intake, e.g., red wine consumption, may improve endothelial function and reduce risk for cardiovascular diseases (Stoclet et al., 2004; Cordova et al., 2005; Opie et al., 2007). Although there have been numerous studies investigating food-derived functional components, the molecular mechanisms for their actions remain to be determined. We found that several natural food-derived components, such as resveratrol, activate peroxisome proliferator-activated receptors (PPARs) and suppress expression of cyclooxygenase (COX)-2. These two properties targeted specifically to PPARs and COX-2 will be important for evaluating food-derived functional components. This review discusses the effects of food-derived functional components on PPARs and COX-2, and evaluates novel functions of these compounds in relation to prevention of lifestyle-related diseases.

Linkage Between PPAR and COX-2

PPARs are members of a nuclear receptor family of ligand-dependent transcription factors (Mangelsdorf et al., 1995). The PPAR subfamily comprises three isotypes, PPARα, β/δ and γ, which play various roles in lipid and carbohydrate metabolism, cell proliferation and differentiation, and inflammation; they are considered molecular targets in the prevention of lifestyle-related diseases (Michalik et al., 2006; Sonoda et al., 2008). For example, PPARα agonists, such as fibrates and thiazolidine derivatives, are used to treat dyslipidemia and diabetes, respectively. Moreover, eicosapentaenoic acid, a natural ligand for PPARα, has been used as a hypolipidemic drug and has been reported to lower plasma and liver cholesterol levels in a PPARα-dependent manner (Sugiyama et al., 2008).

PPARs are also involved in the control of COX-2 expression. COX is the rate-limiting enzyme in prostaglandin (PG) biosynthesis, and its activity is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, indomethacin and ibuprofen (Fig. 1). COX has two isoforms, COX-1 that is expressed constitutively in most cells and COX-2, which is undetectable in most normal tissues. COX-2 is induced by inflammatory stimuli such as endotoxins.
Resveratrol

We firstly found that resveratrol (3, 5, 4'-trihydroxystilbene, Fig. 3) is an activator of PPARs and suppressor of COX-2. Among the food-derived components that have been characterized, resveratrol is one of the most promising, and there is an accumulation of data from experimental studies regarding its actions (Lastra and Villegas, 2005; Baur and Sinclair, 2005; Nakata et al., 2012). Resveratrol, a phytoalexin and antioxidant polyphenolic compound found in red wine and various plant products, has long been suspected to have cardio-protective effects and to contribute to the so-called “French paradox” (i.e., the relatively low incidence of coronary heart disease in France compared to other developed countries with comparable diets; (St. Leger et al., 1979; Renaud and Lorgeril, 1992; Ferrieres, 2004)). Previous studies have investigated the effects of resveratrol on activation of SIRT1, an NAD+-dependent protein deacetylase (Howitz et al., 2003; Baur et al., 2006; Lagouge et al., 2006; Feige et al., 2008; Pfluger et al., 2008), since resveratrol has been reported to act as a calorie restriction (CR) mimetic with potential anti-aging and anti-diabetogenic properties. There is controversy, however, as to whether resveratrol is a direct activator of SIRT1 (Kaeberlein et al., 2005; Dai et al., 2010; Pacholec et al., 2010). Recently, cAMP-dependent phosphodiesterase (PDE) was reported as a novel direct target of resveratrol (Park et al., 2012), although this data is not conclusive. We demonstrated that resveratrol suppressed COX-2 expression in 184B5/HER-transformed mammary epithelial cells (Subbaramaiah et al., 1998), and activated PPARα, β/δ and γ in cell-based reporter assays using bovine arterial and lipopolysaccharide (LPS), suggesting that it plays a role in inflammation (Simmons et al., 2004; Smith, 2008; Koki et al., 2002). In addition, growing evidence indicates that COX-2 expression is regulated differently among cell types, and that it also plays a key role in tumorigenesis (Eberhart et al., 1994; Oshima et al., 1996), development (Morham et al., 1995; Dinchul et al., 1995; Lim et al., 1997; Williams et al., 2000), and circulatory homeostasis (Dubios et al., 1998; Grosser et al., 2006). The PGD₂, metabolite 15-deoxy-∆_{12,14}-PGJ₂ (15d-PGJ₂) was identified as a potent natural ligand of PPARγ (Forman et al., 1995; Kliewer et al., 1995). We previously reported that 15d-PGJ₂ suppressed LPS-induced expression of COX-2 in differentiated, macrophage-like U937 cells, but not in vascular endothelial cells, and that expression of COX-2 was regulated by a negative feedback loop mediated through PPARγ, particularly in macrophages (Inoue et al., 2000) (Fig. 1). Similarly, the PPARα agonist fenofibrate has been reported to inhibit interleukin-1-induced COX-2 expression in smooth muscle cells (Staels et al., 1998). These findings indicate that PPARs participate in cell type-specific control of COX-2 expression (Fig. 2). From these results, we hypothesized that an interaction between activation of PPARs and suppression of COX-2 expression is important in the evaluation of the effects of food-derived functional ingredients.
endothelial cells (BAEC) (Inoue et al., 2003; Tsukamoto et al., 2010). Additionally, in a study using PPARα-knockout mice, resveratrol treatment (20 mg/kg body weight for 3 days) was shown to protect the brain from ischemic stroke through a PPARα-dependent mechanism in mice, indicating that resveratrol activates PPARs in vivo (Inoue et al., 2003). These findings have been corroborated by reports that PPARα mediates some of the effects of CR (Corton et al., 2004). Furthermore, we showed in a previous study that vaticanol C, a resveratrol tetramer, activates PPARα and PPARβ/δ in vitro and in vivo, but has no effect on SIRT1 activation (Tsukamoto et al., 2010). We also found that a 4-week intake of resveratrol led to upregulation of hepatic expression of SIRT1 in wild type, but not PPARα knockout mice (submitted). Polyphenolic compounds such as apigenin, chrysin (Liang et al., 1999; Woo et al., 2005), and humulon (Yamamoto et al., 2000) suppress COX-2 expression and activate PPARα and/or γ (Liang et al., 2001; Yajima et al., 2004). These findings suggest that various food-derived components have effects on PPARs and COX-2 that are comparable to those of resveratrol. Similar to 15d-PGJ2, it is possible that COX-2 expression is regulated by PPAR agonism exerted by dietary components (Fig. 2).

Carvacrol, a Component of Thyme Oil

We screened various food-derived components for their effects on PPARs and COX-2. First, we evaluated commercially available oils derived from 21 kinds of plants, each at a concentration of 0.01%, for their suppressive effect on the COX-2 promoter in BAEC (Inoue et al., 1995). We found that LPS-induced COX-2 promoter activity was suppressed by thyme, clove, rose, eucalyptus, fennel, and bergamot oils in descending order of activity, whereas no suppression of COX-2 promoter activity was observed with castor, corn, cottonseed, fusel, lavender, lemon, linseed, olive, orange, palm, safflower, sesame, soybean, or turpentine oils. Among these essential oils, thyme oil exhibited the strongest effects and showed dose-dependent (0.002 – 0.008%) suppression of LPS-induced COX-2 promoter activity. In addition, in our cell-based assay using BAEC (Tsukamoto et al., 2010), thyme, rose, clove, and bergamot oils had PPARα agonistic activity, and thyme oil also had PPARγ agonistic activity. Remarkably, the range in which thyme oil exerted dose-dependent activation of PPARα and γ was the same as that observed for suppression of LPS-induced COX-2 promoter activity (Hotta et al., 2010).

The main constituents of thyme oil were carvacrol, p-cymene, and linalyl acetate. Carvacrol (Fig. 3), a monoterpenic phenol, suppressed COX-2 promoter activity with a dose dependency of 200 and 400 μM, indicating that carvacrol is the major component of thyme oil involved in suppression of LPS-induced COX-2 promoter activity. However, COX-2 promoter activity was only marginally suppressed in the absence of the PPARγ expression vector; but the suppressive effect was statistically significant in the presence of the PPARγ expression vector in BAEC, indicating that carvacrol-mediated suppression is partly PPARγ dependent. The concentration of carvacrol that activated PPARα and γ was the same as that which suppressed activity of the COX-2 promoter. These concentrations of carvacrol are similar to the concentrations of thyme oil that led to activation of PPARα and γ. Moreover, carvacrol induced mRNA expression of the PPARα-dependent carnitine palmitoyltransferase (CPT) 1 gene in U937 cells. These results indicate that carvacrol is both an activator of PPARα and γ and a suppressor of COX-2 promoter activity. In BAEC, thymol, a structural isomer of carvacrol, but not p-cymene, dehydroxy-carvacrol, activated PPARα and γ. Similarly, p-cymene, did not suppress LPS-induced COX-2 promoter activity, whereas thymol did. In addition, carvacrol and thymol, but not p-cymene, exhibited weak activation of PPARβ/δ. These results suggest that the hydroxyl group of carvacrol is essential for both the activation of PPARα and γ and the suppression of COX-2 promoter activity (Hotta et al., 2010).

Recently, carvacrol was reported to be an agonist of transient receptor potential (TRP) V3, a thermosensitive ion channel expressed predominantly in the skin and neural tissues (Xu et al., 2006; Vogt-Eisele et al., 2007; Vriens et al., 2008). The EC50 of carvacrol for TRPV3 was 490 μM, similar to the effective concentration for PPARs and COX-2 in our study (Table 1). Moreover, thymol, but not p-cymene, is also an agonist of TRPV3 (EC50 = 860 μM), suggesting a structural requirement for a hydroxyl group, which is similar to our results on the effects on COX-2 and PPARα (Table 1).

Citral, a Component of Lemongrass Oil

Lemongrass is a widely used herb, particularly in Southeast Asia and Brazil, where it is used as a food flavoring, a perfume, and for its medicinal properties. Tea or essential

<table>
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<tr>
<th>Chemical</th>
<th>PPAR (EC50)</th>
<th>TRP channel (EC50)</th>
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<tbody>
<tr>
<td>Carvacrol</td>
<td>~0.2 mM (α, γ) 0.49 mM (TRPV3)</td>
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<tr>
<td>Thymol</td>
<td>~0.2 mM (α, γ) 0.86 μM (TRPV3)</td>
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<tr>
<td>Citral</td>
<td>~0.1 mM (α) 33.5 μM (TRPM8) 0.2 mM (TRPA1)</td>
<td></td>
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<tr>
<td>Geraniol</td>
<td>~0.2 mM (α, γ) 5.9 μM (TRPM8) 102 μM (TRPV1)</td>
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<tr>
<td>Citronellol</td>
<td>~0.2 mM (α, γ) 43 μM (TRPV1)</td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td>~0.2 mM (α, γ) 1 ~ 10 μM (TRPV1)</td>
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oil made from lemongrass is popularly used for analgesic and anti-inflammatory purposes. Lemongrass oil has been reported to inhibit the expression of inflammatory cytokines, such as IL-1β and IL-6, in peritoneal macrophages (Sforcin et al., 2008), and to possess promising antitumor activity and cause loss of tumor cell viability (Sharma et al., 2009). With our established assay system (Hotta et al., 2010), we demonstrated that lemongrass oil (0.004%) activated PPARα and PPARγ. Moreover, we found that LPS-induced COX-2 promoter activity was suppressed by lemongrass oil at similar doses that activated PPARγ (Katsukawa et al., 2010).

Citral, a mixture of tautomers geranial (trans-citral) and neral (cis-citral) (Fig. 2), was identified as the major component of lemongrass oil by GC and GC-MS analysis. Citral activated PPARα and PPARγ at doses of 100 and 200 μM, respectively, which is similar to the concentration of citral in 0.004% lemongrass oil. These results indicated that citral was a major component of lemongrass oil that mediated dual activation of PPARα and γ. Moreover, citral had a statistically significant suppressive effect on COX-2 promoter activity (100 and 200 μM) in the presence, but not in the absence of the PPARγ expression vector, indicating that citral suppressed COX-2 promoter activity in a PPARγ-dependent manner. We also confirmed that the suppressive effect on COX-2 promoter activity and activation of PPARγ were observed with a similar concentration range of citral. Citral induced mRNA expression of the PPARα-responsive CPT1 and PPARγ-responsive fatty acid binding protein (FABP) 4 genes in U937 cells. Furthermore, LPS-induced COX-2 mRNA and protein were suppressed by citral. Collectively, these results indicate that citral is both a dual activator of PPARα and γ and a suppressor of COX-2 expression in U937 cells (Katsukawa et al., 2010).

There were several differences between citral and carvacrol, although they had similar effects on PPAR and COX-2. Citral was a more potent activator of PPARα than carvacrol, whereas activation of PPARγ was similar between the two compounds. In addition, citral was a more potent suppressor of COX-2 promoter activity than carvacrol. Our data suggest that citral may also suppress COX-2 promoter activity in a PPARγ-independent manner, since activation of PPARγ was similar with citral and carvacrol. Citral has been reported to suppress the expression of inducible nitric synthase (iNOS) gene by LPS, and suppress the DNA-binding activity of the NF-kB site of the iNOS gene (Lee et al., 2008). Moreover, it has been shown to activate several TRP thermosensitive channels with different affinities: that is TRPM8 (EC50 = 33.5 μM) and TRPA1 (EC50 = 200 μM) (Stotz et al., 2008) (Table 1). Since TRP channels have been reported to be involved in inflammation and cancer (Chaouki et al., 2009), it is possible that activation of TRPM8 by citral is linked to our findings.

**Citronellol and Geraniol, Components of Rose Oil**

Rose oil is one of the most widely used essential oils in perfumes and cosmetics. This oil has been reported to possess a wide range of physiological activities, including analgesic, hypnotic, and anti-inflammatory properties. In our established cell-based assay (Hotta et al., 2010), we found that 0.01% rose oil activated PPARα and γ and that LPS-induced COX-2 promoter activity was suppressed in the presence of 0.01% rose oil (Katsukawa et al., 2011).

The major components of rose oil (0.01%) were citronellol (212 μM), geraniol (179 μM), and nerol (40 μM) (Fig. 2). Citronellol (100 – 400 μM) exhibited PPARα activation, indicating higher PPARα agonistic activity than geraniol, the cis-isomer of nerol. In contrast, citronellol and geraniol displayed similar activation of PPARγ (200 – 400 μM). Nerol did not activate PPARα or γ. These results indicate that citronellol and geraniol were the major components involved in the activation of PPARα and γ by rose oil. Furthermore, citronellol displayed more potent activation of PPARα than geraniol, possibly due to the replacement of the geraniol double bond with the single bond of citronellol, indicating that different ligand-binding pockets may exist between PPARα and γ. Within the concentration range for PPAR activation, citronellol suppressed PPARγ-dependent COX-2 promoter activity, whereas geraniol suppressed PPARγ-independent COX-2 promoter activity. These results demonstrate that citronellol and geraniol were the main components responsible for rose oil-induced suppression of COX-2 promoter activity, but that they exert their actions by potentially different mechanisms. Citronellol and geraniol had remarkably varied effects on PPARα and COX-2 expression. This may be important for an evaluation of the differing ligand specificities of PPARα and γ and the differing mechanisms that mediate suppression of COX-2 expression at the molecular level (Katsukawa et al., 2011).

Recently, citronellol and geraniol were reported to also act as agonists for TRPV1 (Ohkawara et al., 2010). The EC50 of citronellol and geraniol for TRPV1 were 43 μM and 102 μM, respectively (Table 1). It is known that TRPV1 is activated by capsaicin, the main pungent ingredient in red peppers (Caterina et al., 1997). This may be important for understanding the anti-inflammatory and anti-lifestyle-related disease properties of these chemicals in addition to carvacrol and citral.

**Conclusion**

We found that several natural food-derived components activate PPARs and suppress COX-2 expression. By target-
ing this evaluation to PPARs and COX-2, we identified resveratrol as an activator of PPARs and suppressor of COX-2 expression. Carvacrol, citral, citronellol and geraniol, key components in essential oils, also activated PPARs and suppressed COX-2 expression. These results may be important for understanding the anti-inflammatory and anti-lifestyle-related disease properties of these natural components, although further studies in vivo will be necessary to address the physiological significance of the findings. The screening system utilizing receptor assays for PPARs and COX-2 may be applicable for studying the functionality of food-related materials.

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