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Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferator-activated receptor-γ by flavonoids in mouse macrophages

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Abstract Peroxisome proliferator-activated receptor (PPAR)γ transcription factor has been implicated in anti-inflammatory response. Of the compounds tested, apigenin, chrysin, and kaempferol significantly stimulated PPARγ transcriptional activity in a transient reporter assay. In addition, these three flavonoids strongly enhanced the inhibition of inducible cyclooxygenase and inducible nitric oxide synthase promoter activities in lipopolysaccharide-activated macrophages which contain the PPARγ expression plasmids. However, these three flavonoids exhibited weak PPARγ agonist activities in an in vitro competitive binding assay. Limited protease digestion of PPARγ suggested these three flavonoids produced a conformational change in PPARγ and the conformation differs in the receptor bound to BRL49653 versus these three flavonoids. These results suggested that these three flavonoids might act as allosteric effectors and were able to bind to PPARγ and activate it, but its binding site might be different from the natural ligand BRL49653. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Peroxisome proliferator-activated receptor-γ; Flavonoid; Inflammation; Cyclooxygenase; Nitric oxide synthase; Lipopolysaccharide

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor gene family [1]. PPARs bind to specific response elements as heterodimers with the retinoid X receptor and activate transcription in response to a variety of endogenous and exogenous ligands, including certain polyunsaturated fatty acids, arachidonic acid metabolites [2], and some anti-diabetic drugs [3] and non-steroidal anti-inflammatory drugs (NSAIDs) [4]. Currently, PPARs subfamily has been defined as PPARα, PPARβ (also called PPARδ and NUC1) and PPARγ. Three PPAR isoforms differ in their tissue distribution and ligand specificity [5]. PPARα is predominantly expressed in tissues exhibiting high catabolic rate of fatty acids (heart, liver, and kidney), whereas PPARδ expression is ubiquitous, and its physiological role is unclear. PPARγ is expressed predominantly in adipose tissue, the adrenal gland, spleen, large colon and the immune system [6–9]. Several lines of evidence indicated that PPARγ plays an important role in regulating adipocyte differentiation and glucose homeostasis [10]. Both PPARα and PPARγ have been shown that also have anti-inflammatory actions through activating by arachidonic acid metabolites. PPARα bind and be activated by leukotriene B4 [11], and the levels are induced at the transcriptional level by anti-inflammatory glucocorticoids [12]. PPARγ are activated by the prostaglandin D_2 metabolite 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (15d-PGJ_2) and synthetic anti-diabetic thiazolidinedione drugs (e.g. BRL49653 and ciglitizone) and resulted in negatively regulating the expression of pro-inflammatory genes, and suppressing tumor cell growth [13–16]. Furthermore, both PPARα and PPARγ are activated by a number of NSAIDs, such as indomethacin [4]. Recently, the PPARγ agonists have been considered to inhibit production of monocyte inflammatory cytokines and the expression of inducible nitric oxide synthase (iNOS) [17,18].

The flavonoids are a diverse family of chemicals commonly found in fruits and vegetables. Flavonoids are plant polyphenolic compounds, which comprise several classes including flavonols, flavanones, flavanols and flavans. Epidemiological studies have shown that the consumption of vegetable, fruits and tea is associated with a decreased risk of cancer and cardio-vascular diseases, and flavonoids are believed to play an important role in preventing these diseases [19]. Numerous numbers of this family have anti-carcinogenic [20], anti-inflammatory [21], cytostatic [22], apoptotic [18], anti-oxidant [23], anti-angiogenic [24] and estrogenic [25] activities. Several reports have also shown that flavonoids are potent modulators of both the expression, and activities of specific cytochrome P450 genes/proteins [26]. These data indicate that certain flavonoids have attracted attention as possible chemoprotective or chemotherapeutic agents.

NSAIDs such as aspirin, sodium salicylate, and indomethacin exert their anti-inflammatory effects in part by inhibition of inhibitor κB (IκB) kinase-β (IKK-β), thereby preventing activation by nuclear factor-κB (NF-κB) of genes involved

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; NSAIDs, non-steroidal anti-inflammatory drugs; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2; COX, cyclooxygenase; PGE_2, prostaglandin E_2; iNOS, inducible nitric oxide synthase; NO, nitric oxide; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; IκB, inhibitor κB; IKK, IκB kinase; IFN-γ, interferon-γ; Gst, glutathione *S*-transferase

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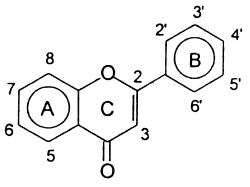
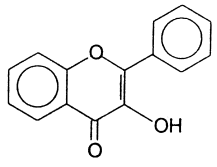
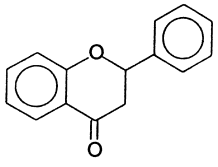
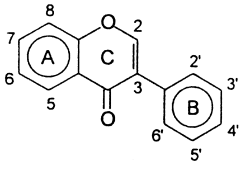
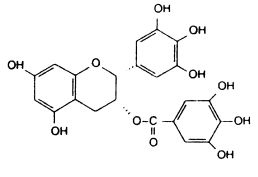
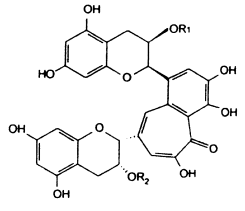
Table 1
Chemical structures of the various flavonoids tested for the induction of PPARγ activity in RAW246.7 cells

Chemical formula	Name	Substitution							
		5	6	7	8	2'	3'	4'	5'
Flavones									
	Flavone	H	H	H	H	H	H	H	H
	5-Methoxyflavone	OCH_3	H	H	H	H	H	H	H
	7,8-Dihydroxyflavone	H	H	OH	OH	H	H	H	H
	Chrysin	OH	H	OH	H	H	H	H	H
	Apigenin	OH	H	OH	H	H	H	OH	H
	Luteolin	OH	H	OH	H	H	OH	OH	H
	Tangeretin	OCH_3	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H
Flavonols									
	3-Hydroxyflavone	H	H	H	H	H	H	H	H
	Galangin	OH	H	OH	H	H	H	H	H
	Kaempferol	OH	H	OH	H	H	H	OH	H
	Fisetin	H	H	OH	H	H	OH	OH	H
	Morin	OH	H	OH	H	OH	H	OH	H
	Quercetin	OH	H	OH	H	H	OH	OH	H
	Myricetin	OH	H	OH	H	H	OH	OH	OH
Flavonol glycoside	Rutin	OH	H	OH	H	H	OH	OH	H
		(3: OR; R: Rhamnosylglucoside)							
Flavanones									
	Pinocembrin	OH	H	OH	H	H	H	H	H
	Naringenin	OH	H	OH	H	H	H	OH	H
	Isosakuranetin	OH	H	OH	H	H	H	OCH_3	H
	Eriodictyol	OH	H	OH	H	H	OH	OH	H
	Hesperetin	OH	H	OH	H	H	OH	OCH_3	H
Flavanone glycoside	Naringin	OH	H	OR	H	H	H	OH	H
		(R: Rhamnosylglucoside)							
Isoflavones									
	Genistein	OH	H	OH	H	H	H	OH	H
	Biochanin A	OH	H	OH	H	H	H	OCH_3	H
Flavan-3-ol		**Theaflavins**							
									
(-)-Epigallocatechin-3-gallate		Theaflavin-3,3'-digallate (R=Galloyl)							

in the inflammatory response [27]. However, indomethacin and several other NSAIDs (fenoprofen, ibuprofen, and flufenamic acid) are also PPARγ ligands and block production of inflammatory cytokines in human monocytes [17]. In addition, Ricote et al. [13] also demonstrated that treatment of peritoneal macrophages with 15d-PGJ_2 or several synthetic PPARγ ligands reduce the expression of iNOS by interferon-γ (IFN-γ) and inhibited induction of gelatinase B and scavenger receptor A gene transcription in response to phorbol ester stimulation. Recently, we reported that apigenin and related flavonoids could suppress the transcriptional activity of cyclooxygenase (COX)-2 and iNOS in part through inhibition of IKK activity [28]. The current study was designed to determine whether the anti-inflammatory effects of flavonoids were correlative with their activation of PPARγ.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (LPS) (*Escherichia coli* 0127:B8), flavone, 5-methoxyflavone, 7,8-dihydroxyflavone, apigenin, 3-hydroxyflavone, kaempferol, morin, quercetin, myricetin, rutin, genistein, indomethacin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Chrysin, luteolin, tangeretin, galangin, fisetin, pinocembrin, naringenin, isosakuranetin, eriodictyol, hesperetin, naringin, and biochanin A were purchased from Extrasynthese Inc. (Genay, France). Mouse IFN-γ was purchased from R&D systems Inc. (Minneapolis, MN, USA). Two kinds of tea polyphenols, (−)-epigallocatechin-3-gallate and theaflavin-3,3′-digallate were purified as previously described [29].

2.2. Cell culture

The mouse macrophage cell lines RAW264.7 (ATCC, T1B71) were cultured as previously described [28]. Thioglycollate-elicited peritoneal macrophages were obtained from specific pathogen-free female Balb/c mice as previously described [30]. For all assays except the luciferase assay, cells were plated in 60 mm dishes at 5×10^6 cells/dish and allowed to grow for 18–24 h. Treatment with vehicle (0.1% DMSO), test compounds and/or LPS or IFN-γ were carried out under serum-free conditions.

2.3. Determination of prostaglandin E_2 (PGE_2) and nitrite

The cultured medium of control and treated cells were collected, centrifuged, and stored at −70°C, until tested. The level of PGE_2 released into culture media was quantified using a specific enzyme immunoassay according to the manufacturer's instructions (Amersham). The nitrite concentration in the cultured medium was measured as an indicator of NO production according to the Griess reaction [28].

2.4. Plasmids

The PPARγ expression plasmid and AOx-TK reporter plasmid were generously provided by Professor Christopher K. Glass (California University) [13].

The mouse iNOS promoter plasmid was generously provided by Professor Charles J. Lowenstein (Johns Hopkins University) [31]. The mouse COX-2 promoter plasmid containing a 1035 bp fragment, −966 to +70 relative to the transcription start, and constructed as previously described [28]. To generate the pGEX-2T-PPARγ LBD chimeric receptor expression plasmid, cDNA encoding the ligand binding domains (LBD) of the mouse PPARγl(amino acids 174–475) were amplified by polymerase chain reaction and subcloned into the pGEX-2T expression plasmid. Transient cotransfection and luciferase activity assay using these plasmids were performed as described previously [28].

2.5. Ligand binding assay

The glutathione *S*-transferase (Gst)–PPARγ LBD was expressed in JM109 *E. coli* [32] and the fusion proteins were bound to the glutathione Sepharose-4B beads according to the manufacturer's instructions (Pharmacia Biotech). For competition binding assay, 10 μl of glutathione Sepharose-4B beads containing 0.1 μg of Gst–PPARγ LBD chimeric protein were incubated with or without unlabeled flavonoids at 4°C for 12 h in buffer containing 10 mM Tris (pH 7.4), 50 mM KCl, 10 mM dithiothreitol and proteinase inhibitors, then added [^{3}H]BRL49653 (specific activity, 60 Ci/mmol) for additional 8 h. Bound [^{3}H]BRL49653 was precipitated from free radioactivity by centrifugation, and washed three times with PBS. The beads containing [^{3}H]BRL49653 were collected and quantitated by liquid scintillation counting.

2.6. Limited protease digestion assay

The protease digestion assays were performed by the method of Allen et al. [33], with some modification. The PPARγ expression plasmid [13] was used to synthesize ^{35}S-radiolabeled PPARγ in a coupled transcription/translation system according to the protocol of the manufacturer (Promega). Approximately 5 μl of the transcription/translation reactions was preincubated with 1 μl of tested compounds for 20 min at 25°C. Trypsin was added and allowed to proceed for 10 min at 25°C, then terminated by the addition of SDS sample loading buffer and boiling for 8 min. The products of the digestion were separated by electrophoresis through a 12% SDS–polyacrylamide gel. Labeled PPARγ was visualized by autoradiography.

3. Results

3.1. Apigenin, chrysin, and kaempferol activated PPARγ in macrophages

A series of flavonoids including flavone, 5-methoxyflavone, 7,8-dihydroxyflavone, apigenin, 3-hydroxyflavone, kaempferol, morin, quercetin, myricetin, rutin, genistein, chrysin, luteolin, tangeretin, galangin, fisetin, pinocembrin, naringenin, isosakuranetin, eriodictyol, hesperetin, naringin, and biochanin A, tea polyphenols (Table 1), and indomethacin were first tested with regard to their activation effects on PPARγ in RAW264.7 cells. As RAW264.7 cells express very low levels of PPARγ [13] and required transfection of a PPARγ expression plasmid. The cells therefore allowed a direct assessment of the role of PPARγ in mediating the effects of these flavonoids on macrophage gene expression. The PPARγ expression plasmid was cotransfected into RAW264.7 cells with a reporter construct containing three copies of the acyl CoA oxidase PPAR responsive element upstream of the thymidine kinase (TK) promoter driving luciferase gene expression. In the absence of a cotransfected PPARγ expression plasmid, treatment of RAW264.7 macrophages with the tested flavonoids at 10 μM had little effect on activation of PPARγ. However, when a PPARγ expression plasmid was cotransfected into the cells, apigenin, chrysin, and kaempferol significantly induced the PPARγ 8.13-, 5.60- and 7.66-fold, respectively ($P<0.05$) (Fig. 1A). The positive control of indomethacin (100 μM) strongly induced the PPARγ activity 13.34-fold which was compared with the mock experiment. Apigenin, chrysin, and kaempferol increased the PPARγ activity in a dose-dependent manner, with the EC_{50} of approximately 5 μM, 10 μM, and 10 μM, respectively (Fig. 1B). However, there was a cytotoxic effect in RAW264.7 cells with 20 μM of apigenin and a decrease in the activation of PPARγ. Flavone, 7,8-dihydroxyflavone, 3-hydroxyflavone, luteolin, galangin, genistein, biochanin A also increased the PPARγ activities when RAW264.7 cells were transfected with the expression plasmid of PPARγ. However, the induction folds of these flavonoids showed no significant difference in activation of PPARγ compared with control RAW264.7 cells that were transfected with PPARγ expression plasmid (Figs. 1A and 2, 2.74-fold). The other tested flavonoids were unable to activate the PPARγ, and the data were not shown in Fig. 1A.

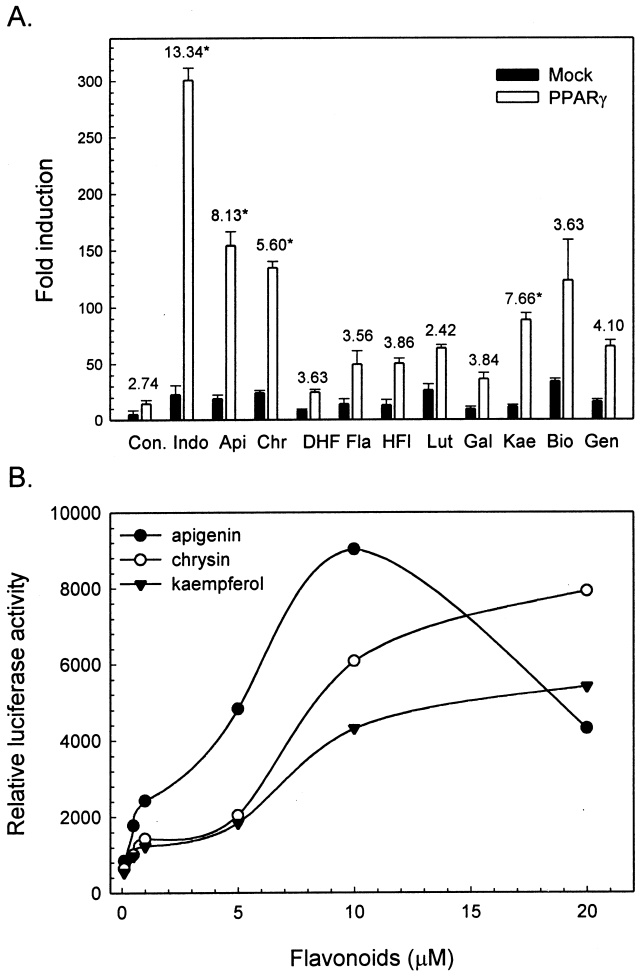


Fig. 1. Effects of apigenin, chrysin, and kaempferol on the activation of PPARγ in cells. RAW264.7 cells were cotransfected with the AOx-TK reporter plasmid and PPARγ expression plasmid (open squares) or PPARγ mock plasmid (closed squares) and β-galactosidase expression vector (pcDNA lacZ) as internal control. A: Transfected cells were treated with 10 μM of flavonoids, or 100 μM of indomethacin for 18 h, and cell extracts subsequently assayed for luciferase and β-galactosidase activities as described in Section 2. The values were represented as means ± S.E.M. of triplicate tests. The numbers were obtained from ratios of the induction fold of transfected PPARγ expression to mock expression and indicated in the figure. $*P < 0.05$ (Student's t-test) versus control (2.74). B: Transfected cells were treated with various concentrations of apigenin (closed circles), chrysin (open circles), and kaempferol (closed triangles) for 18 h, and cells extracts assayed for luciferase and β-galactosidase activities as described above. Con., control; Indo, indomethacin; Api, apigenin; Chr, chrysin; DHF, 7,8-dihydroxyflavone; Fla, flavone; HFl, 3-hydroxyflavone; Lut, luteolin; Gal, galangin; Kae, kaempferol; Bio, biochanin A; Gen, genistein.

3.2. Apigenin, chrysin and kaempferol enhanced the inhibition of COX-2 and iNOS promoters' activities in a PPARγ-dependent manner

RAW264.7 cells was transiently transfected with the reporter plasmids of COX-2 or iNOS and both promoters' activities were markedly increased when RAW264.7 cells were treated with LPS (Fig. 2). Both promoters' activities were inhibited by concurrent treatment of the cells with apigenin, chrysin and kaempferol. Moreover, transfection of PPARγ expression plasmid enhanced the inhibitory effects of these three flavonoids (Fig. 2A,B). The results suggested that apigenin, chrysin and kaempferol inhibited the promoters' activities of COX-2 and iNOS genes partially through PPARγ pathways.

3.3. Apigenin, chrysin, and kaempferol bound with PPARγ and induced conformational change in PPARγ

We next sought to determine whether these three flavonoids activated PPARγ through direct interaction with the PPARγ receptor. The abilities of these flavonoids to bind to PPARγ were assessed in a competition binding assay using [^{3}H]BRL49653 and the glutathione Sepharose beads containing Gst–PPARγ LBD fusion protein. As shown in Fig. 3, [^{3}H]BRL49653 bound specifically and saturably to Gst–PPARγ LBD beads with a K_d of 8 nM (Fig. 3A,B). No binding was detected in control Gst–PPARγ LBD beads (data not shown). Apigenin, chrysin, and kaempferol competed with [^{3}H]BRL49653 for binding to the PPARγ LBD in a dose-dependent manner, with an IC_{50} of approximately 50 μM. Pro-

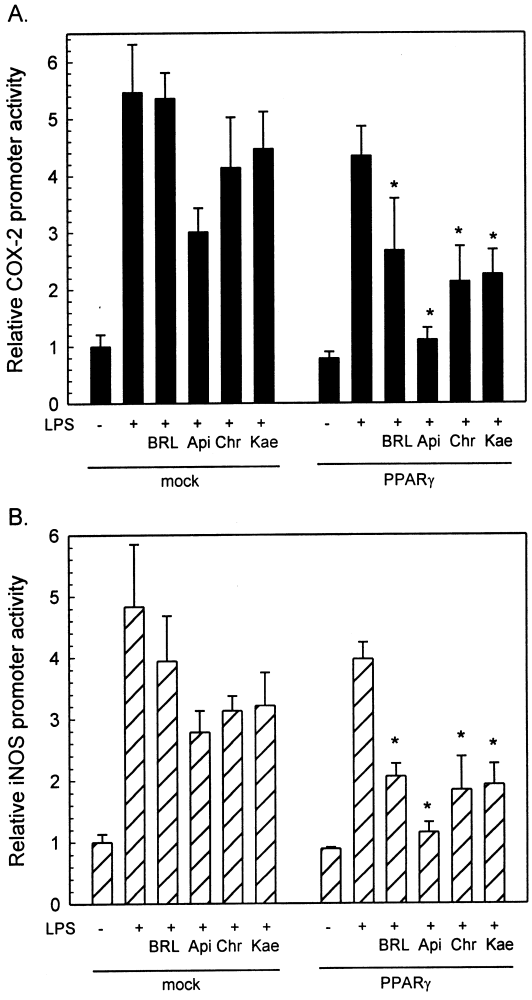


Fig. 2. Apigenin, chrysin and kaempferol enhanced the inhibition of the promoter activities of COX-2 and iNOS in a PPARγ-dependent manner. RAW264.7 cells were cotransfected with a COX-2 (A) or iNOS (B) promoter-luciferase reporter plasmid, pcDNA lacZ internal control vector, and either a PPARγ expression plasmid or mock plasmid as indicated. Cells were treated with LPS (50 ng/ml) and BRL49653 (20 μM), or various flavonoids (10 μM) for 18 h, and extracts subsequently assayed for luciferase and β-galactosidase activities as described in Section 2. The values were represented as means ± S.E.M. of triplicate tests. $*P < 0.05$, compared with the individual test of mock expression (Student's t-test).

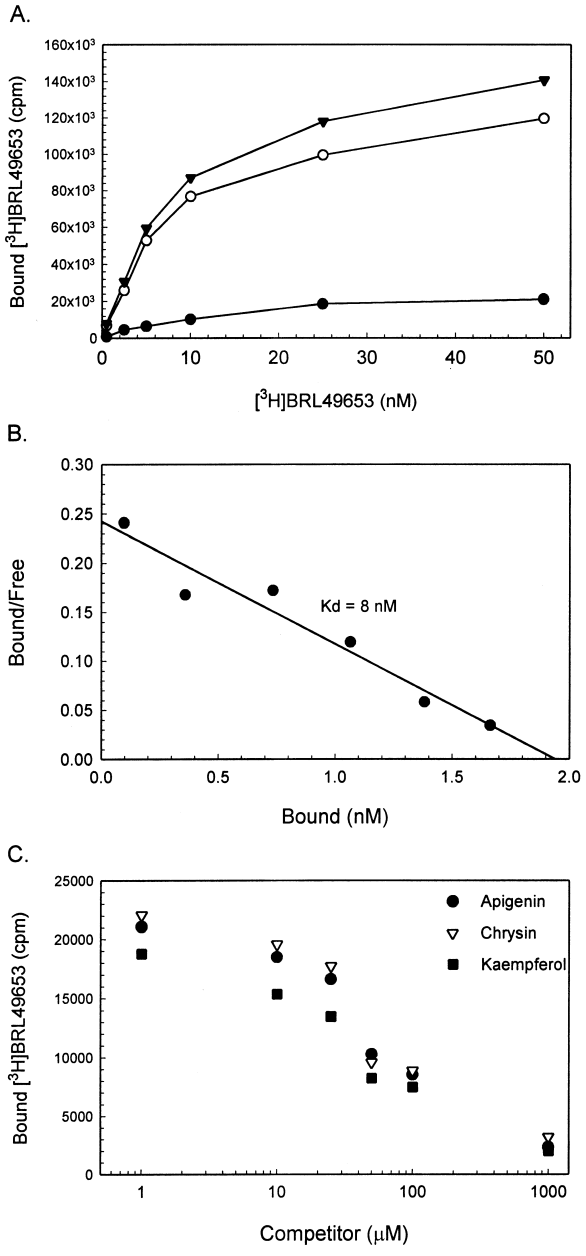


Fig. 3. The binding capacity of apigenin, chrysin, and kaempferol to PPARγ in vitro. A: Glutathione Sepharose beads containing the Gst–PPARγ LBD fusion protein were incubated with increasing concentrations of [^{3}H]BRL49653. Non-specific binding was determined using 500-fold excess of unlabeled BRL49653. Closed triangles, total binding; closed circles, non-specific binding; open circles, specific binding. B: Scatchard analysis of specific binding data from A. Linear regression yielded a K_d of 8 nM. Similar results were obtained in three independent experiments performed in duplicate. C: Competition binding analysis was performed with Sepharose–GST–PPARγ LBD beads and 5 nM [^{3}H]BRL49653 in the presence of various concentrations of apigenin (closed circles), chrysin (open triangles), or kaempferol (closed squares) as unlabeled competitors. Similar results were obtained in two independent experiments performed in duplicate.

teolytic analysis have been used for several experiments to demonstrate that ligands of nuclear receptor can, upon binding, specifically alter the conformation of the receptor [33,34]. This conformational change was reflected by the increased resistance of the receptor to partial digestion by proteases. To determine if there were conformational differences in PPARγ bound to these three flavonoids, a limited trypsin digestion on a [^{35}S]methionine-labeled PPARγ was performed. As shown in Fig. 4, incubation of PPARγ with increasing concentrations of trypsin in the absence of ligand led to the complete digestion of PPARγ. In contrast, BRL49653 induced a stronger protection of the 22, 29 and 30 kDa fragments. These three flavonoids binding yielded 29 and 30 kDa protected fragments, especially a 30 kDa band. These results indicated that these three flavonoids were able to bind to PPARγ and flavonoid-bound PPARγ had a distinct trypsin digestion pattern compared to the BRL49653-bound receptor.

4. Discussion

Flavonoids are naturally occurring plant polyphenols found in abundance in the diets rich in fruit, vegetables and plant-derived beverages such as tea. The PPARγ ligands share certain structural characteristics including a lipophilic backbone and an acid moiety, usually a carboxylate. Although flavonoids only have a similar lipophilic backbone, several flavonoids also bind to PPARγ in vitro (Fig. 4). Several reports have shown that treatment of various fibroblast and mesenchymal stem cell lines with PPARγ ligands, including 15d-PGJ_2, the anti-diabetic drugs, and several NSAIDs, promotes their efficient conversion to adipocytes [3,4,35–37]. However, treatment of C3H10T1/2 stem cells with 10 μM of apigenin, chrysin, and kaempferol failed to promote adipocytes differentiation as indicated by Oil Red O staining (data not shown). Northern blot analysis indicated that PPARγ expression levels were not induced in response to treatment with these three flavonoids in fibroblast cells (data not shown). As we know, adipocyte differentiation required forced expression of PPARγ and was significantly enhanced in the presence of PPARγ activators [38]. For example, treatment of C3H10T1/2 cells with BRL49653 increased PPARγ expression levels approximately three-fold, and resulted in efficient adipocyte differentiation [3]. In addition, while flavonoids were potent inhibitors of several kinases involved in signal transduction, mainly protein kinase C [39] and tyrosine kinase [40,41]. However, adipocyte differentiation is characterized by a coordinate increase in adipocyte-specific gene expression through activating of gene transcription. Therefore, they were insufficient to initiate the adipogenic signaling cascade in a mesenchymal stem cell line. Devchand et al. [11] proposed that leukotriene B4, an agonist of the related receptor PPARα, has anti-inflammatory activity. The flavonoids might also activate the PPARα and have anti-inflammatory effects, but PPARα is not expressed in activated macrophages.

Of the tested flavonoids (Table 1), the groups of flavanones and flavan-3-ol were inefficient on activation of PPARγ. This result suggested that the C2–C3 double bond of C ring was essential for their activation of PPARγ. Some flavonoids of flavones, flavonols and isoflavones groups were able to activate PPARγ. The activation of PPARγ seems to be dependent on the number and the position of hydroxyl residues. The hydroxyl residues of the 5 and 7 positions of the A ring and 4′ position of the B ring were important factors for activation of PPARγ, such as in apigenin, chrysin and kaempferol. However, the additional hydroxyl residue of the 3′ position of the B ring resulted in a decrease of the activation of PPARγ, such as in luteolin and quercetin.

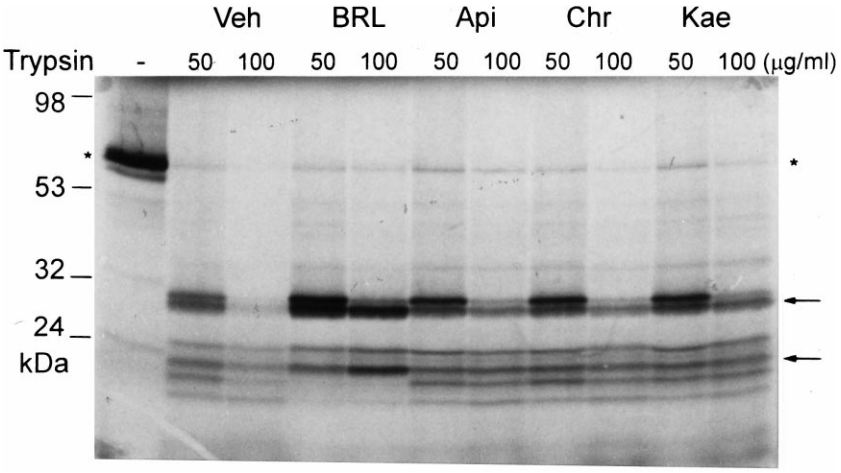


Fig. 4. Apigenin, chrysin and kaempferol induced a protease-resistant conformation of PPARγ. [^{35}S]PPARγ was synthesized in vitro in a coupled transcription/translation system. The receptor was preincubated with ethanol (Veh), apigenin (Api), chrysin (Chr) or kaempferol (Kae), then added to dH_2O or increasing concentration of trypsin. Digestion products were analyzed by SDS–PAGE followed by autoradiography. Asterisks and arrows indicate the full-length PPARγ and trypsin-resistant fragment of PPARγ, respectively.

These three flavonoids were able to activate PPARγ in a transient reporter assay, with an EC_{50} of approximately 5–10 μM. However, they needed a higher concentration to bind to Gst–PPARγ in an in vitro competitive binding assay (Fig. 3). The high concentration of IC_{50} (50 μM) suggested that these three flavonoids might not directly bind to PPARγ or bind to PPARγ in the other sites. A limited protease digestion assay (Fig. 4) indicated that PPARγ has a conformational difference in the receptor bound to the three flavonoids versus natural ligand BRL49653. These results suggested that these three flavonoids might act as allosteric effectors, and were able to bind to PPARγ and activate it, but its binding site might be different from the natural ligand BRL49653. Based on the different binding kinetics, it may interpret the fact that these three flavonoids did not promote differentiation of C3H10T1/2 stem cells to adipocyte. Our previous studies have shown that apigenin was able to inhibit IKK activity and prevent the activation of NF-κB, and then suppress the promoter activities of COX-2 and iNOS [28]. Therefore, apigenin was a more stronger inhibitor of COX-2 and iNOS promoters activities than BRL49653 in the absence and presence of transfected PPARγ expression plasmid (Fig. 2). However apigenin was more effective in the presence of PPARγ than absence of PPARγ in inhibition of COX-2 and iNOS promoters. These results indicated that these three flavonoids inhibited the expression of COX-2 and iNOS partially through activating PPARγ. In RAW264.7 cells, the base level of PPARγ protein was very low, so BRL49653 could not activate it to suppress the activities of COX-2 and iNOS promoters in the absence of transfected PPARγ expression plasmid.

Our results suggested that certain flavonoids could activate PPARγ to inhibit the protein expression of COX-2. Indomethacin was able to inhibit COX activity without affecting the protein levels of PPARs at lower concentration [42]. At a higher concentration, we thought that anti-inflammatory activity of indomethacin might be also mediated through activation of PPARγ followed by inhibition of COX expression, since indomethacin also acts as a PPARγ agonist, promoting adipocyte differentiation.

Our previous studies demonstrated that the anti-inflammatory properties of apigenin might be mediated through inhibition of IKK activity. In this study, we showed that apigenin was also an efficacious activator of PPARγ which regulated inflammatory responses. These results suggested that apigenin, chrysin, and kaempferol were possible activators of PPARγ, and might have therapeutic applications in inflammatory diseases, such as atherosclerosis and rheumatoid arthritis. These findings also provide a significant molecular basis for explaining how dietary flavonoids are active in preventing cancer and inflammation.

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