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(−)-Catechin promotes adipocyte differentiation in human bone marrow mesenchymal stem cells through PPARγ transactivation

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ABSTRACT

Green tea intake has been shown to confer various health benefits to patients suffering from metabolic disorders. Here, we studied the effect of several major green tea polyphenols on adipocyte differentiation in human bone marrow mesenchymal stem cells (hBM-MSCs) and compared it to the effect of representative antidiabetic drugs. (−)-Catechin was the most potent of the eight green tea polyphenols evaluated in promoting adipocyte differentiation in hBM-MSCs, and this effect was dose-dependent. (−)-Catechin increased the mRNA levels of various adipogenic markers, such as adiponectin, peroxisome proliferator-activated receptor gamma (PPARγ), FABP4, and LPL, as measured during adipocyte differentiation in hBM-MSCs. In addition, (−)-catechin upregulated the secretion of adiponectin in hBM-MSC culture. Using a reporter gene assay and a competitive ligand binding study, (−)-catechin also significantly activated PPARγ in a dose-dependent fashion; however, (+)-catechin, the enantiomer of (−)-catechin, was not effective as a PPARγ agonist, which seems to imply that the effect of (−)-catechin on PPARγ is stereospecific. In conclusion, our data suggest that (−)-catechin promotes adipocyte differentiation and increased sensitivity to insulin in part by direct activation of PPARγ, which could be at the basis of the observed pharmacological benefits of green tea intake in reducing the risk of type 2 diabetes.

1. Introduction

Green tea is a widely consumed beverage in Asia and has been considered to confer considerable health benefits to patients suffering from metabolic diseases, such as type 2 diabetes [1,2]. Type 2 diabetes is characterized by resistance to insulin and by incomplete metabolic profiles, including impaired glucose tolerance in skeletal muscle, adipose tissue, and liver [3,4].

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Abbreviations: hBM-MSCs, human bone marrow mesenchymal stem cells; PPARγ, peroxisome proliferator-activated receptor-γ; TZDs, thiazolidinediones; FABP4, fatty acid binding protein 4; LPL, lipoprotein lipase; (−)-EGCG(−), -epigallocatechin-3-gallate; (−)-EC(−), -epicatechin; (−)-EGC(−), -epigallocatechin; (−)-ECG(−), -epicatechin gallate; (−)-GCG(−), -gallocatechin-3-gallate; (−)-CG(−), -catechin-3-gallate; (−)-GC(−), -gallocatechin.

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To understand the cellular and molecular mechanisms of action of green tea in type 2 diabetes, green tea or its polyphenols have been studied using various experimental designs. According to a recent clinical study, green tea consumption is inversely associated with a risk for developing type 2 diabetes [5]. It has also been reported that green tea intake improves glucose tolerance and insulin sensitivity in healthy men [6,7]. In mouse models, green tea administration results in the increase of energy expenditure and the promotion of fat oxidation [8,9]. Furthermore, catechins, which are a major class of green tea polyphenols, were shown to not only diminish serum lipid levels, but also to reduce blood pressure in Sprague Dawley and Obese Zucker rats [10].

Adiponectin is an adipokine exclusively secreted by adipocytes, which regulates energy metabolism mainly by increasing insulin sensitivity [12,13]. Clinically, plasma levels of adiponectin are significantly decreased in patients with type 2 diabetes, and the magnitude of the reduction is strongly correlated with the severity of insulin resistance in muscle and liver [11]. Therefore, adiponectin is regarded as being a crucial tool for the diagnoses of type 2 diabetes, and possibly also as being a clinical marker of the effect of antidiabetic drugs. Recently, a change in the serum adiponectin/leptin ratio was validated as a diagnostic parameter for type 2 diabetes [14,15]. Many antidiabetic drugs improve the alteration of the serum adiponectin/leptin ratio by increasing adiponectin secretion in adipocytes and also by improving insulin sensitivity. For instance, the antidiabetic thiazolidinediones, which are known as ligands of the peroxisome proliferator-activated receptor gamma (PPARγ), were shown to significantly increase adiponectin expression during adipocyte differentiation [16,17].

We have previously examined the effects of several green tea polyphenols on the expression of adiponectin in murine 3T3-L1 pre-adipocytes, and found that (−)-catechin increased adiponectin secretion in these cells [18]. Although the suppression of Kruppel-like factor 7 by (−)-catechin may be associated with this effect, the exact molecular mechanism that explains the increase of adiponectin in 3T3-L1 cells remains unknown. Recently, human bone marrow mesenchymal stem cells (hBM-MSCs) have been explored as an alternative to murine pre-adipocytes (such as 3T3-L1 cells) in the study of human metabolic diseases [19]. The neogenesis of adipocytes in adult tissues is a complex process, starting with the commitment of mesenchymal precursor cells to pre-adipocytes, to the establishment of the adipogenic lineage, and ending in their terminal differentiation. Therefore, hBM-MSCs have been proposed as a cellular model to study the commitment of human precursor cells to the adipogenic lineage, which would contribute to the advancement of our understanding of human obesity and diabetes [19–21].

In the present study, we evaluated the applicability of hBM-MSCs to study the molecular mechanisms of the effect of catechins (present in green tea) on type 2 diabetes. We found that (−)-catechin not only promoted adipocyte differentiation in hBM-MSCs in a fashion similar to antidiabetic drugs, but also increased the expression levels of several adipogenic markers, including adiponectin, LPL, FABP4, and PPARγ. Interestingly, using a luciferase reporter assay and a competitive binding assay, we observed that (−)-catechin stereospecifically transactivated PPARγ. We therefore demonstrated that (−)-catechin upregulated the process of adipocyte differentiation in hBM-MSCs by acting as a PPARγ agonist.

2. Materials and methods

2.1. Cell culture and differentiation

Human bone marrow mesenchymal stem cells were purchased from Lonza, Inc. (Walkersville, MD). hBM-MSCs were grown in Dulbecco Modified Eagle's Medium (DMEM) with low glucose (1 g/L) containing 10% fetal bovine serum (FBS) and supplemented with antibiotics and Glutamax™ (Invitrogen, Carlsbad, CA). To induce adipocyte differentiation, the growth medium was replaced with DMEM containing a high glucose concentration (4.5 g/L) and supplemented with 10% FBS, Glutamax™, 10 μg/ml insulin, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (IDX adipogenic condition). Insulin, IBMX, glibenclimide, and troglitazone were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO). (−)-Catechin, (+)-catechin, (−)-epigallocatechin-3-gallate (EGCG); (−)-epicatechin (EC); (−)-epigallocatechin (EGC); (−)-epicatechin gallate (ECG); (−)-gallocatechin-3-gallate (GCG); (−)-catechin-3-gallate (CG); (−)-gallocatechin (GC) were also purchased from Sigma–Aldrich Chemical Company (St. Louis, MO). hBM-MSCs were grown in the IDX condition and treated with each catechin, troglitazone, or glibenclamide, respectively, every 2 days, for 6 days or for 12 days.

2.2. Oil Red O staining and hematoxylin staining

Adipocyte differentiation was assessed using an Oil Red O stain (Sigma–Aldrich Chemical Co., St. Louis, MO) as an indicator of intracellular lipid accumulation. After hBM-MSC differentiation to adipocytes, cells were rinsed twice with phosphate-buffered saline (PBS), fixed with 10% formalin in PBS (pH 7.4) for 1 h, and then washed with 60% isopropanol, before being allowed to dry completely. hBM-MSCs were stained with 0.2% Oil Red O reagent for 10 min at room temperature, and washed with H_2O four times. Each hBM-MSC sample was eluted with 100% isopropanol for 10 min and absorbance was measured at 500 nm using a spectrophotometer. To visualize the nucleus, hBM-MSCs were counterstained with hematoxylin reagent for 2 min and the washed twice with H_2O. The level of adipocyte differentiation was observed using an inverted phase-microscope.

2.3. Measurement of adiponectin levels

For quantitative determination of adiponectin in cell culture supernatants, a Quantikine™ immunoassay kit was used (R&D Systems, Minneapolis, MN). The media treated with green tea polyphenols and antidiabetic drugs were centrifuged for 5 min at 1000 g and the supernatants were subsequently diluted for use in the quantification reaction. Adiponectin levels were determined according to the manufacturer's instructions.

2.4. Real-time quantitative RT-PCR

Total RNA from hBM-MSCs allowed to undergo differentiation via a 7-day exposure to the adipogenic condition was isolated

using Trizol (Invitrogen, Carlsbad, CA), followed by a purification step using the Qiagen RNeasy kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. The RNA concentration of each sample was determined by spectrophotometry at 260 nm. The integrity of each RNA sample was evaluated using the Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA). Two micrograms of total RNA from each sample were reverse transcribed to the corresponding cDNA using the Superscript Reverse Transcriptase (RT) II kit (Invitrogen, Carlsbad, CA). The RT reaction was stopped by adding Tris–EDTA buffer (pH 8.0) to a total of 100 μl of cDNA solution, to be used in subsequent assays. Quantitative measurements of various adipogenic markers in each sample were carried out using the Assays-on-Demand™ Gene Expression kits (Applied Biosystems, Foster City, CA). Each kit consists of a FAM™ dye-labeled TaqMans® MGB probe and primer pair for specific marker genes. cDNA samples were analyzed for adiponectin (ADIPOQ, Hs00605917_m1), fatty acid binding protein 4 (FABP4, Hs00609791_m1), peroxisome proliferator activated receptor gamma (PPARγ, Hs00233423_m1), and lipoprotein lipase (LPL, Hs01012571_m1).

2.5. Luciferase reporter gene assay

To perform a luciferase reporter gene assay, CV-1 cells were seeded into 24-well plates and cultured for 24 h prior to transfection with a DNA mixture containing PPARγ responsive elements (PPRE)-luciferase reporter plasmid (0.3 μg), pcDNA3-hPPARγ (30 ng), and internal control plasmid pRL-SV-40 (10 ng), using the TransFast™ transfection reagent (Promega, Madison, WI). After 24 h of transfection, cells were treated for an additional 24 h with troglitazone (10 μM) or the indicated concentrations of (−) catechin or (+) catechin. The activity of luciferase in each cell lysate was measured using the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI), according to the manufacturer's instructions. Firefly and *Renilla* luciferase activities were determined using Wallac Victor II luminometer (PerkinElmer, Waltham, MA). To calculate the relative luciferase activities, the firefly luciferase activities were normalized by the corresponding *Renilla* luciferase activities.

2.6. Fluorescence polarization assay

Fluorescence polarization was measured using the Polar-Screen™ PPAR competitor assay kit (Invitrogen, Carlsbad, CA). Briefly, the competitors [(+) catechin, (−) catechin] were serially diluted in reaction buffer in a black 384-well plate (Cliniplate 384, Thermo LabSystems, Finland), followed by the addition of purified PPARγ-ligand binding domain and fluorescent PPAR ligand (Fluormone™ PPAR green). The samples were incubated at room temperature for 2 h and fluorescence polarization was measured using a Safire™ microplate reader (Tecan, Salzburg, Austria) set at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

2.7. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and were presented as means ± S.D. of at least three independent experiments. The threshold of significance was set at $P < 0.05$.

3. Results

3.1. (−)-Catechin is the most potent of the green tea polyphenols tested to promote adipocyte differentiation in hBM-MSCs

The IDX adipogenic condition includes a three-hormone cocktail (insulin, dexamethasone, and IBMX) and strongly induces the differentiation of nearly all murine 3T3-L1 cells into adipocytes; however, the IDX condition is not as efficient in promoting adipocyte differentiation as in hBM-MSCs [19,20]. Therefore, to increase the adipogenic potential of human cells, adipogenic-promoting agents such as troglitazone are added to the adipogenic IDX cocktail. Because many antidiabetic drugs promote adipocyte differentiation and increase insulin sensitivity, the adipocyte differentiation of hBM-MSCs has been used to evaluate antidiabetic compounds [16,17].

In order to examine the effect of various green tea polyphenols on adipocyte differentiation, we treated hBM-MSCs with (−)-catechin (C), (−)-EGCG, (−)-EC, (−)-EGC, (−)-ECG, (−)-GCG, (−)-CG, and (−)-GC, at a concentration of 10 μM each in the IDX condition medium (Fig. 1). In addition, we compared the adipogenic effects of green tea polyphenols with those induced by troglitazone (2 μM), a PPARγ agonist, and glibenclimide (10 μM), a sulfonylurea-type antidiabetic drug. Troglitazone concentrations higher than 10 μM led to cytotoxicity in hBM-MSCs in culture (data not shown). Incubation with 10 μM of (−)-catechin significantly increased

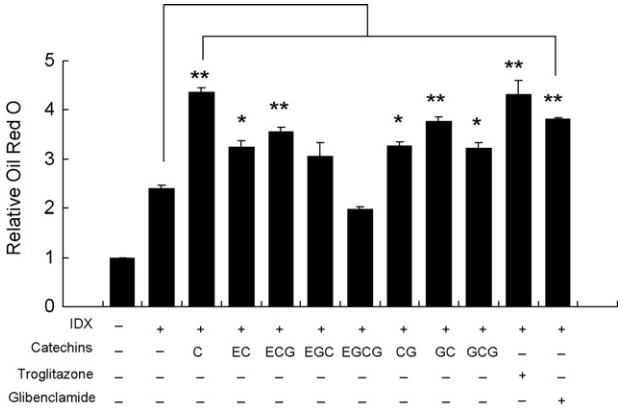

Fig. 1 – Effect of various green tea polyphenols on adipocyte differentiation in hBM-MSCs.
hBM-MSCs were grown in the IDX condition and treated with various types of catechins (10 μM each of (−)-catechin (C), EC, ECG, EGC, EGCG, CG, GC, GCG) every 2 days for 12 days. As positive controls we used 10 μM of glibenclimide and 2 μM of troglitazone. Lipid droplets in adipocytes were stained with Oil Red O dye and then quantified by densitometry at 500 nm using a spectrophotometer. Data were normalized by setting the IDX (−) control as 1 (mean ± S.D.). *P < 0.05 and **P < 0.01, compared with IDX (+).

adipocyte differentiation in hBM-MSCs by 81%, when compared with the IDX condition (Fig. 1). In parallel cell cultures, treatment with 2 μM of troglitazone and 10 μM of glibenclamide enhanced adipocyte differentiation in hBM-MSCs by 80% and 59%, respectively. (−)-ECG and (−)-GC also significantly promoted adipocyte differentiation by 48% and 57%, respectively (Fig. 1). In contrast to the other polyphenols, incubation of cells with 10 μM of (−)-EGCG resulted in a slight decrease in adipocyte differentiation, when compared with the IDX condition. In an independent test, we observed that

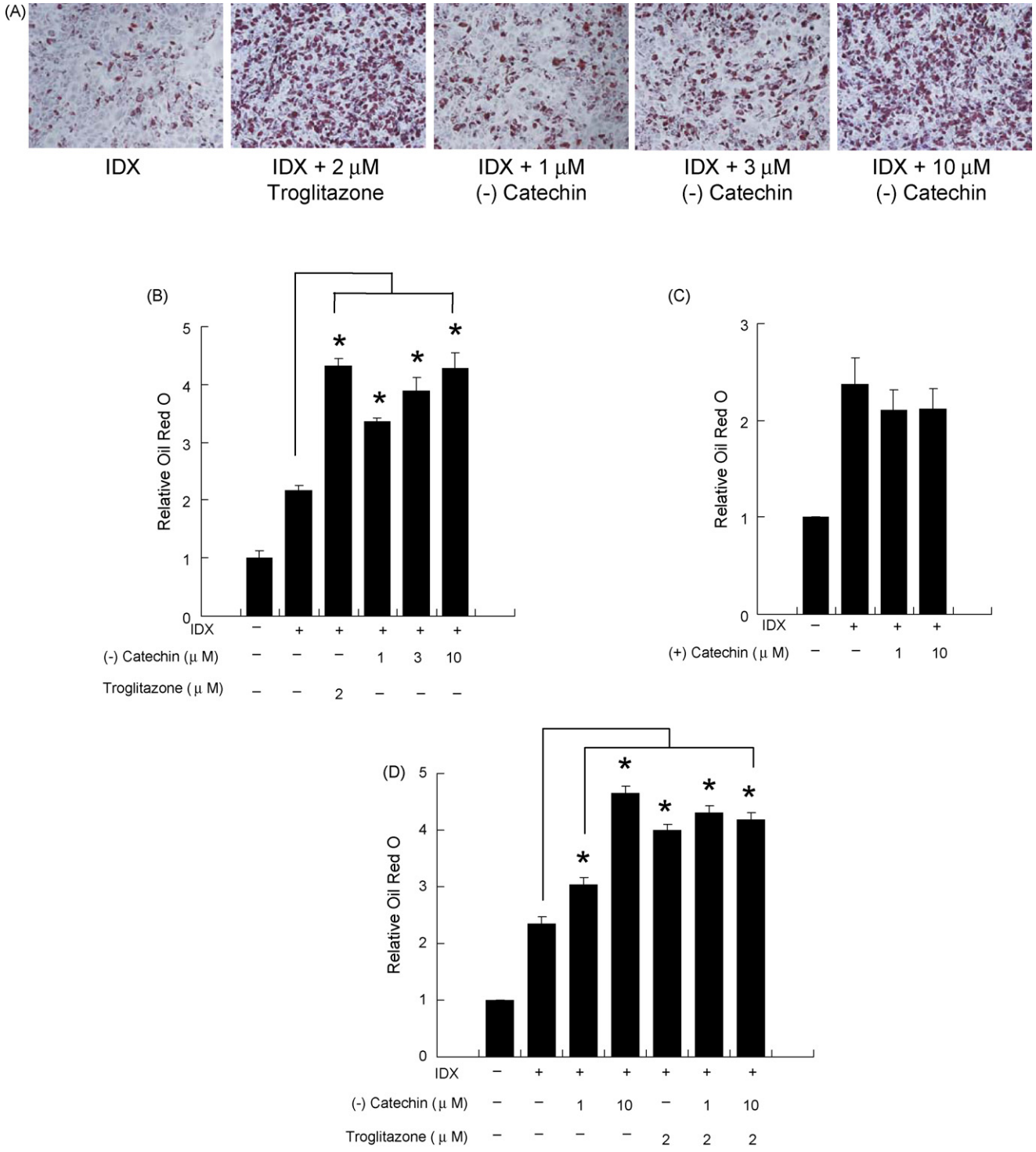

Fig. 2 – Dose-dependent effect of (−)-catechin on adipocyte differentiation in hBM-MSCs.
hBM-MSCs were grown in the IDX condition and treated with (−)-catechin (1, 3, and 10 μM) (A and B), (+) catechin (1 and 10 μM) (C), or (−)-catechin (1 and 10 μM) in the presence or absence of troglitazone (2 μM) (D), every 2 days for 12 days. Lipid droplets in adipocytes were stained with Oil Red O dye and then quantified by densitometry at 500 nm using a spectrophotometer. Data were normalized by setting the IDX (−) control as 1 (mean ± S.D.). *P < 0.05, compared with IDX (+).

50 μM of (−)-EGCG inhibited adipocyte differentiation by 51% (data not shown).

3.2. *(−)-Catechin dose-dependently and stereospecifically promotes the adipocyte differentiation of hBM-MSCs*

We next evaluated the effect of (−)-catechin concentration on the adipocyte differentiation in hBM-MSCs. As shown in Fig. 2A and B, (−)-catechin enhanced adipocyte differentiation in these cells in a concentration-dependent manner. According to previous reports, catechins in green tea possess antioxidant activity [21], which in turn is known to be correlated with an increase in adipocyte differentiation [22,23]. In order to examine the possibility that adipocyte differentiation may be accelerated by the antioxidant radical scavenging effect of (−)-catechin, we tested whether its enantiomer (+)-catechin also has the ability to promote adipocyte differentiation in hBM-MSCs. However, we found that (+)-catechin had no effect (Fig. 2C). This result suggests that (−)-catechin significantly enhances the adipocyte differentiation of hBM-MSCs in a stereospecific manner. However, in the IDX condition including 2 μM of troglitazone, the addition of (−)-catechin did not affect the level of adipocyte differentiation in hBM-MSCs (Fig. 2D). Because there is no synergistic effect between troglitazone and (−)-catechin, it may be possible that either the differentiation potential of hBM-MSCs is already at the maximum level when 2 μM troglitazone are added, or that (−) catechin and troglitazone share a molecular pathway of adipocyte differentiation in hBM-MSCs.

3.3. *Effect of (−)-catechin on the expression of adipogenic markers in hBM-MSCs*

To confirm that (−)-catechin enhances adipocyte differentiation in hBM-MSCs, we measured the transcriptional activities

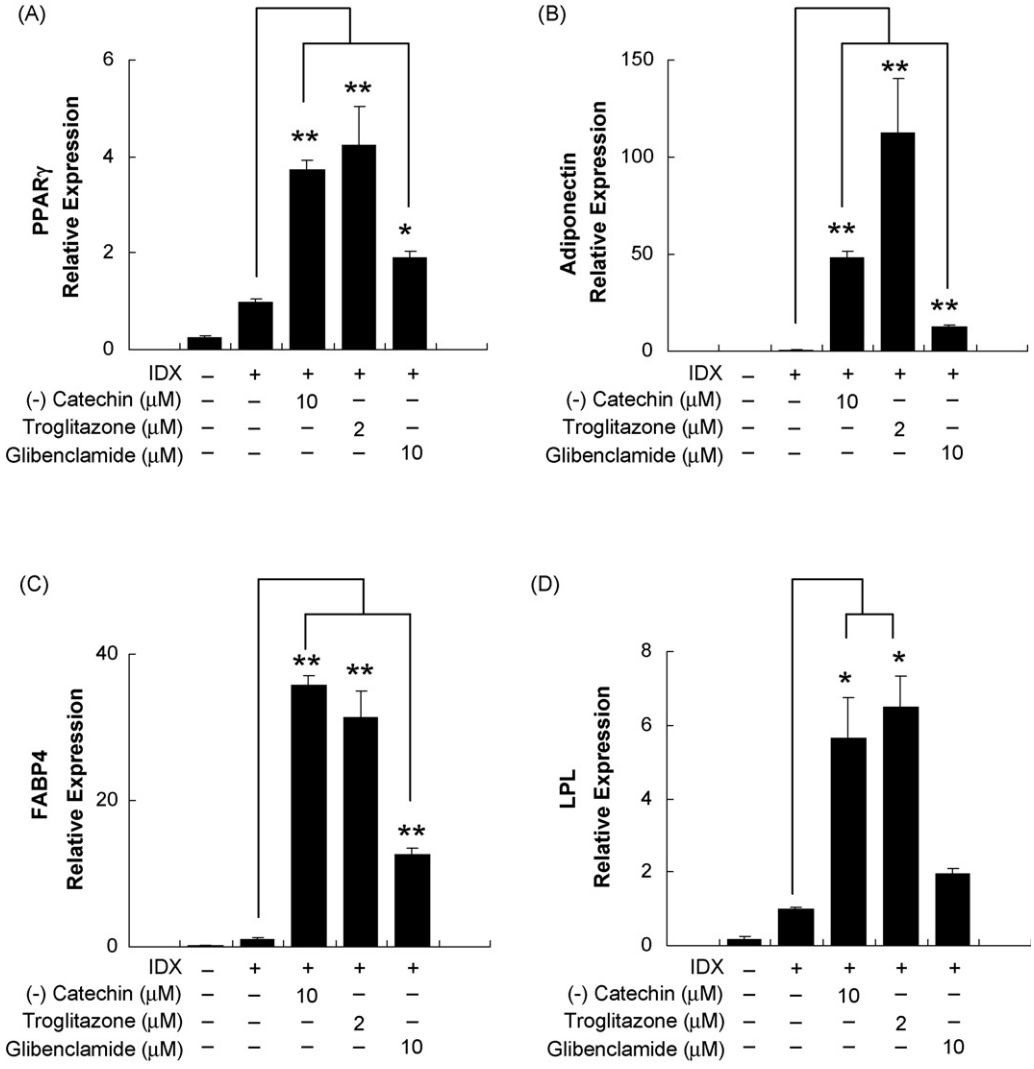

Fig. 3 – Effects of (−)-catechin on the expression of adipocyte differentiation markers.
hBM-MSCs were grown in the IDX condition and treated with (−)-catechin (10 μM) every 2 days for 6 days. Relative expression of PPARγ (A), adiponectin (B), FABP4 (C), and LPL (D) were determined for each sample. Data were calculated using GAPDH as an internal standard and were normalized by setting the IDX (+) as 1 (mean ± S.D.). *P < 0.05 and **P < 0.01, compared with IDX (+).

of the adipocyte specific genes PPARγ, adiponectin, FABP4, and LPL. As shown in Fig. 3, the mRNA level of PPARγ, a key regulator of adipocyte differentiation, was significantly upregulated by the addition of 10 μM of (−)-catechin to the IDX condition, and this effect was as potent as those of 10 μM of glibenclimide (Fig. 3A). The mRNA levels of adiponectin, FABP4, and LPL were also significantly increased by the IDX condition with (−)-catechin (Fig. 3B–D).

Next, we performed ELISA to measure the adiponectin levels in hBM-MSCs cultured in the IDX adipogenic condition treated with (−)-catechin. Consistently with our previous studies in murine 3T3-L1 pre-adipocytes [18], we found that the level of adiponectin was significantly increased in cells treated with (−)-catechin, when compared with cells grown in the IDX condition in the absence of the polyphenol. Moreover, the level of adiponectin was increased by (−)-catechin in a dose-dependent manner. Interestingly, the effect of 10 μM of (−)-catechin on adiponectin secretion was as potent as that of 10 μM of the antidiabetic glibenclimide (Fig. 4).

3.4. *(−)-Catechin promotes adipocyte differentiation in hBM-MSCs directly through activation of PPARγ*

At the earlier stage of adipocyte differentiation, the activation of PPARγ turns on adipogenic signals and results in a phenotypic change from pre-adipocytes or mesenchymal stem cells to differentiated adipocytes containing lipid droplets [24–26]. In order to investigate the molecular mechanism of action of (−)-catechin in promoting adipocyte differentiation in hBM-MSCs, we first performed a luciferase reporter gene analysis to measure the transactivation of PPARγ As shown in Fig. 5A, (−)-catechin significantly induced PPARγ transcriptional activation in a dose-dependent manner. Although treatment with 10 μM of (+)-catechin led to a 2-fold

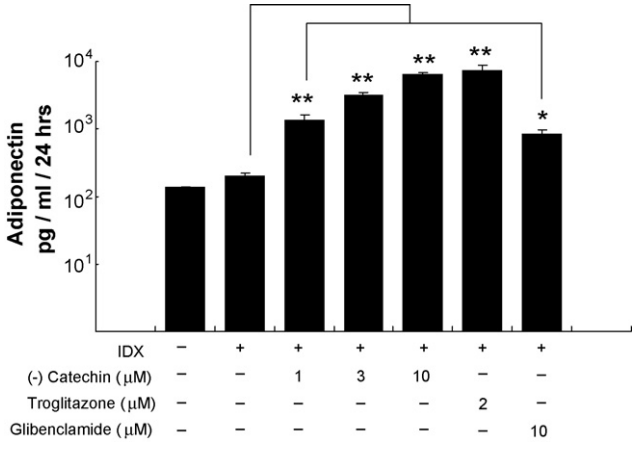

Fig. 4 – Adiponectin induction by (−)-catechin in hBM-MSC culture supernatants.
hMSCs were grown in the IDX condition and treated with (−)-catechin (1, 3, and 10 μM) every 2 days for 12 days. The concentration of the adiponectin accumulated in cell culture supernatants for 24 h after the final medium change at day 12 was measured by ELISA. Data were shown as log scale (mean ± S.D.). *P < 0.05 and **P < 0.01, compared with IDX (+).

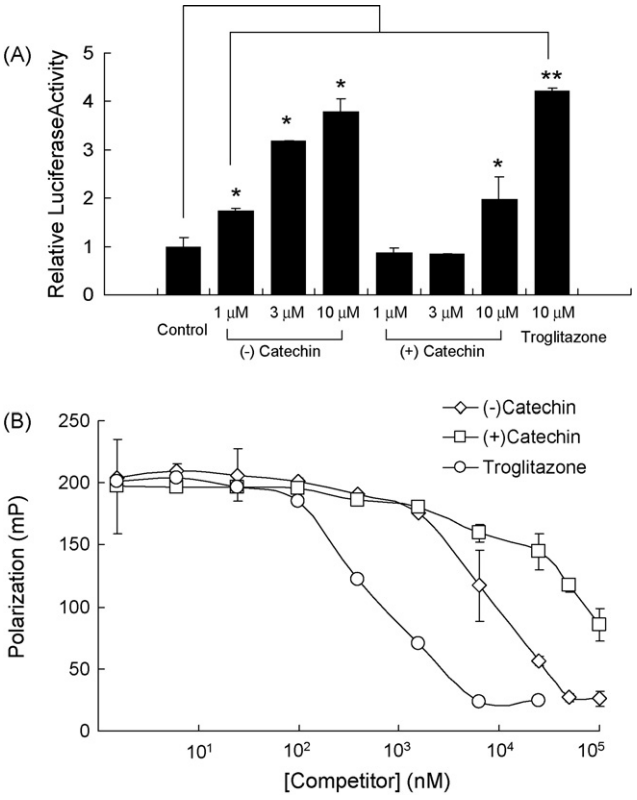

Fig. 5 – Effects of (−)-catechin on transcriptional activation of PPARγ.
(A) CV-1 cells were transiently cotransfected with the PPARγ expression vector and the PPRE-TK-Luciferase reporter, and then treated with vehicle, (−)-catechin/(+)-catechin (1, 3, and 10 μM), or troglitazone (10 μM). (B) Direct binding of (−)-catechin, (+)-catechin, or troglitazone to PPAR-ligand binding domain was assessed using fluorescence polarization. Purified PPARγ-ligand binding domain and fluorescent PPAR ligand (Fluormone™ PPAR Green) were added to different concentrations of troglitazone (○), (−)-catechin (◇), and (+)-catechin (□). The samples were quantified with an excitation wavelength of 485 nm and emission wavelength of 535 nm. Data are presented as mean ± S.D. *P < 0.05, compared with control.

increase in PPARγ transcription, lower concentrations of this molecule had no effect on the transcriptional activity of PPARγ. This result suggests that (−)-catechin promotes the adipocyte differentiation of hBM-MSCs dose-dependently and stereospecifically by enhancing the transcriptional activity of PPARγ.

To determine whether (−)-catechin directly binds to the PPARγ ligand binding site, we performed a receptor ligand binding assay comprising the PPARγ ligand binding domain, a PPAR fluorescent ligand (Fluormone™ PPAR Green), and (−)- or (+)-catechin as competitors. As shown in Fig. 5B, (−)-catechin competitively inhibited the binding of the PPAR fluorescent ligand to the PPARγ ligand binding domain in a concentration-dependent manner (IC_{50}, 9.9 μM). In the same analysis, the IC_{50} of troglitazone and (+)-catechin were 0.7 and 52 μM, respectively. These data suggest that (−)-catechin can

directly bind to the PPARγ ligand binding site and induce adipocyte differentiation in hBM-MSCs via an increase in PPARγ activity.

4. Discussion

A failure in adipocyte differentiation has been suggested as one of the many causes of type 2 diabetes. According to previous reports, adipocytes of type 2 diabetes patients are insulin-resistant and are not capable of accumulating lipids to their full capacity [27–29]. These studies suggest that the surplus of energy must be tightly regulated via the expansion of adipocytes. Otherwise, the inability of adipocytes to expand to store excess calories leads to ectopic accumulation of triglycerides in muscle and liver and results in insulin resistance, the characteristics of type 2 diabetes. Adipocyte differentiation in cell culture has thus been used as a model of insulin insensitivity to study novel antidiabetic drugs [16,17].

The health benefits of green tea intake for type 2 diabetes patients have been assessed by various researchers [5–10]. In the present study, we provide evidence that (−)-catechin is the most potent green tea polyphenol in stimulating adipocyte differentiation in hBM-MSCs, and that (−)-catechin acts as a PPARγ agonist. In addition, (−)-catechin increased adiponectin expression in a dose-dependent manner, which directly supports the hypothesis that (−)-catechin can enhance insulin sensitivity. These effects of (−)-catechin on adipocyte differentiation in hBM-MSCs were as potent as those of the antidiabetic drug glibenclimide. (−)-ECG and (−)-GC also significantly increased adipocyte differentiation and adiponectin levels in hBM-MSCs and may therefore play a role in the antidiabetic effect of green tea. On the other hand, 50 μM of (−)-EGCG, the most abundant green tea polyphenol, significantly inhibited adipocyte differentiation in hBM-MSCs, when compared with a control (data not shown). The anti-adipogenic effect of (−)-EGCG was consistent with previous studies conducted using murine 3T3-L1 pre-adipocytes [30,31]. For instance, (−)-EGCG reduced fatty acid and triglyceride synthesis by inhibiting lipogenic enzymes and significantly downregulated the expression of adipogenic marker genes during adipocyte differentiation. In addition, (−)-EGCG enhanced glucose tolerance by reducing hepatic glucose production and enhanced pancreatic function in db/db mice and obese Zucker rat models [32]. However, in the present study, (−)-EGCG treatment was not associated with significant changes in adiponectin secretion during adipocyte differentiation in hBM-MSCs. Considering that the onset of type 2 diabetes may be partly associated with the failure of adipocytes to differentiate properly, the anti-adipogenic effect of (−)-EGCG in hBM-MSCs described here may dampen the conclusions of previous studies assessing the benefits of green tea intake in type 2 diabetes. Therefore, the interaction between anti-adipogenic green tea polyphenols, such as (−)-EGCG, and pro-adipogenic components, including (−)-catechin, (−)-ECG, and (−)-GC, should be carefully considered in future studies of the health benefits of green tea intake in various metabolic diseases.

It has been well known that PPARγ activation increases the rate and level of adipocyte differentiation in 3T3-L1 pre-adipocytes [16,17]. Although we demonstrated that (−)-catechin directly activated PPARγ, its binding potency to PPARγ was far less than those of conventional glitazone PPARγ agonists (Fig. 5B). In our previous study, the treatment of 3T3-L1 pre-adipocytes with (−)-catechin led only to a slight increase (~17.8%) in adipocyte differentiation measured by Sudan II lipid staining when compared with the IDX condition [18]. However, in the present study, (−)-catechin promoted the adipocyte differentiation of hBM-MSCs to 81% (Fig. 1). As previously mentioned, the IDX adipogenic condition alone is not sufficient to convert human pre-adipocytes or human mesenchymal stem cells into adipocytes, even if this condition is sufficient for the full differentiation of 3T3-L1 pre-adipocytes [19,20]. To induce adipocyte differentiation in human cells, PPARγ agonists such as troglitazone or rosiglitazone have to be added to the IDX condition. When we counted the number of differentiated Oil Red O-stained hBM-MSCs at day 15 after adipogenic stimulation, we found that the IDX condition converted less than 10% of cells into an adipocyte phenotype (data not shown). The level of differentiation of hBM-MSCs in our study was consistent with other reports in the literature [19,20]. Because the presence of PPARγ agonists is not an essential requirement for the full adipocyte differentiation of 3T3-L1 pre-adipocytes, therefore, the effect of PPARγ-activating (−)-catechin on the 3T3-L1 pre-adipocytes in our previous study [18] may be not as much as that on hBM-MSCs (Fig. 1). On the other hand, it is still possible that there are other mechanisms of action for explaining the effect of (−)-catechin working in hBM-MSCs but not in 3T3-L1 pre-adipocytes.

Sulfonlyurea antidiabetic drugs such as glimepiride and glibenclamide are known to stimulate insulin secretion in pancreatic β cells mainly by blocking the conductance of adenosine triphosphate (ATP)-dependent potassium (K_{ATP}) channel via binding to sulfonylurea receptor 1 (SUR1) [33,34]. Interestingly, it has also been reported that glibenclamide antagonized K_{ATP} channel by acting on SUR1 in human adipocytes and upregulated lipogenesis by increasing fatty acid synthase activity [35]. Although glimepiride and glibenclamide were reported to partially increase PPARγ activity, results of the variation of adiponectin levels by treatment with these drugs in 3T3-L1 cells are contradictory [36,37]. It is still unclear whether there is a homologous mechanism acting in 3T3-L1 cells like the interaction between SUR1 and K_{ATP} channel in human adipocytes. On the other hand, we consistently observed that glibenclamide not only enhanced adipocyte differentiation but also increased adiponectin levels in hBM-MSCs. Therefore, our results indicate that hBM-MSCs may be advantageous over murine 3T3-L1 pre-adipocytes as a cell-based model to be used for the evaluation of insulin sensitivity as well as for the development of novel antidiabetic drugs.

According to previous reports, reactive oxygen species inhibit adipocyte differentiation [22], and transgenic mice overexpressing the antioxidant enzyme glutathione peroxidase develop obesity [23]. Therefore, the effect of (−)-catechin on adipocyte differentiation in hBM-MSCs may be partly due to the antioxidant effect of the green tea polyphenol. To evaluate the involvement of an antioxidant effect in our system, we compared (−)-catechin with its stereoisomer

(+)-catechin to test if the latter was also able to promote adipocyte differentiation in hBM-MSCs. However, we found that (+)-catechin did not significantly influence adipocyte differentiation in hBM-MSCs. Furthermore, the IC_{50} value of (+)-catechin was calculated to be about 50 μM in the PPARγ ligand binding assay, but 50 μM of (+)-catechin caused a weak cellular toxicity in hBM-MSCs (data not shown). Therefore, the stereospecificity of the adipogenic-promoting effect of (−)-catechin may exclude the involvement of the antioxidant activity of this green tea polyphenol in adipocyte differentiation. In addition, the stereospecificity of the effect suggests that there may be a direct pharmacological target regulated by (−)-catechin. Using both the luciferase reporter gene and ligand binding assays, we demonstrated that (−)-catechin acted as a PPARγ agonist. Although the IC_{50} value of (−)-catechin (9.9 μM) is higher than that of troglitazone (0.7 μM) as determined in the ligand binding assay, (−)-catechin is structurally different from glitazone antidiabetic drugs and may therefore provide a novel pharmacological target for the development of PPARγ agonists and antidiabetic drugs. Therefore, the structural analysis of the binding properties of (−)-catechin to PPARγ becomes central to the study of the effects of (−)-catechin on adipocyte differentiation and type 2 diabetes.

In conclusion, we described the pharmacological mechanism underlying the effect of green tea intake on adipocyte differentiation in hBM-MSCs. We demonstrated that (−)-catechin stimulates the transcriptional activation of PPARγ, a main regulator of adipocyte differentiation, by directly binding to the receptor, and enhances adiponectin expression, which is known to relieve insulin resistance. Our results therefore suggest that (−)-catechin could potentially be used as a new pharmacophore in the design novel PPARγ agonists to treat type 2 diabetes.

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Conflict of interest

Authors: Dong Wook Shin, Sang Min Lee, Min Jeong Song, Sun Mi Park, Tae Ryong Lee, Joo-Hyun Baik, Han Kon Kim, and Minsoo Noh, are employees of AmorePacific Co.

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