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Kojyl cinnamate ester derivatives promote adiponectin production during adipogenesis in human adipose tissue-derived mesenchymal stem cells

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ABSTRACT

The subcutaneous fat tissue mass gradually decreases with age, and its regulation is a strategy to develop anti-aging compounds to ameliorate the photo-aging of human skin. The adipogenesis of human adipose tissue-mesenchymal stem cells (hAT-MSCs) can be used as a model to discover novel anti-aging compounds. *Cinnamomum cassia* methanol extracts were identified as adipogenesis-promoting agents by natural product library screening. Cinnamates, the major chemical components of *Cinnamomum cassia* extracts, promoted adipogenesis in hAT-MSCs. We synthesized kojyl cinnamate ester derivatives to improve the pharmacological activity of cinnamates. Structure–activity studies of kojyl cinnamate derivatives showed that both the α,β-unsaturated carbonyl ester group and the kojic acid moiety play core roles in promoting adiponectin production during adipogenesis in hAT-MSCs. We conclude that kojyl cinnamate ester derivatives provide novel pharmacophores that can regulate adipogenesis in hAT-MSCs.

Subcutaneous fat tissue gradually thins with age.[1] This is associated with the increased risk of skin injury, a deteriorated skin function to regulate body temperature, and impaired dermal elasticity.[2,3] Human mesenchymal stem cells (hMSCs) exist in virtually all human mesenchymal tissues and can differentiate into adipocytes.[4] Therefore, chemical compounds that promote adipocyte differentiation in MSCs resident in subcutaneous fat tissue may counteract the aging-related functional decreases. Human adipose tissue-mesenchymal stem cells (hAT-MSCs) can be used as a model system to study adipocyte differentiation in fat tissue.[4] In murine pre-adipocytes like 3T3-L1 cells, treatment with an adipogenic cocktail consisting of insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX) (the IDX condition), can induce nearly all pre-adipocytes to acquire differentiated adipocyte phenotypes.[5,6] However, the IDX condition cannot induce the adipocyte differentiation of all the hMSCs tested in two dimensional cell cultures.[6,7] Approximately ten percent of hMSCs in cell culture have an adipocyte phenotype. When sulfonylurea-type antidiabetic drugs like glibenclamide and peroxisome proliferator-activated receptor γ (PPARγ) agonists like troglitazone are added to hMSCs cultured in the IDX condition, the fraction of differentiated adipocytes is increased. Chemical compounds with the potential to promote adipocyte differentiation in hAT-MSCs can be evaluated by testing them in combination with the IDX adipogenic cocktail in cell culture.[6,8] To discover novel pharmacophores capable of regulating the thickness of subcutaneous fat tissue, we have screened natural product libraries using hAT-MSCs as an adipocyte differentiation model. Adiponectin can be measured to quantitatively evaluate the level of adipocyte differentiation in hAT-MSCs.[9] A preliminary screening showed that ethanol extracts of *Cinnamomum cassia* (Ramulus Cinnamomi and Cortex Cinnamomi) promoted adipocyte differentiation (Fig. 1A).[10] The major chemical components of *Cinnamomum cassia* include cinnamaldehyde, cinnamic acid, and eugenol (Fig. 1B).[11] After determining the highest non-cytotoxic concentrations for cinnamaldehyde, cinnamic acid, and eugenol on hAT-MSCs (Fig. 1C–E), we evaluated the effects of the major chemical components in *Cinnamomum cassia* on adipocyte differentiation. The results (Fig. 1F) showed that supplementation with

Abbreviations: hAT-MSCs, human adipose tissue-derived mesenchymal stem cells; PPARγ, peroxisome proliferator-activated receptor gamma.

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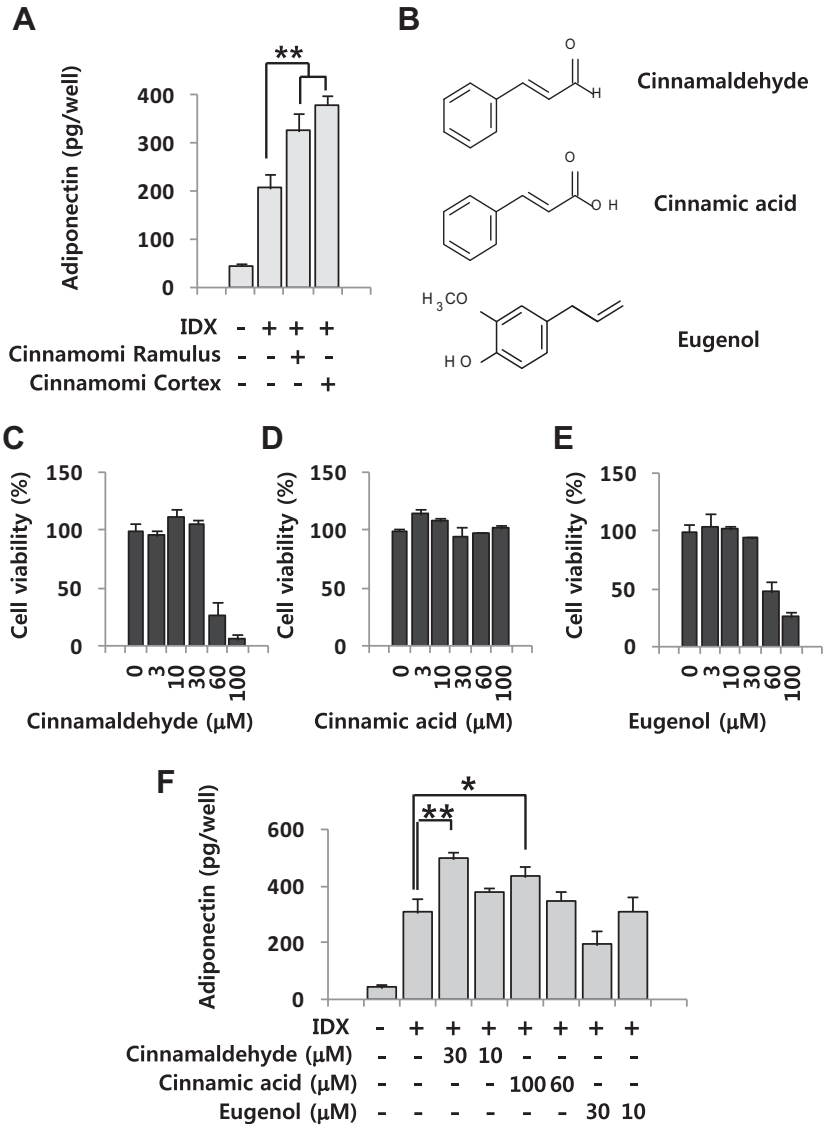

Figure 1. Effects of *Cinnamomum cassia* extracts on adipogenesis in hAT-MSCs. hAT-MSCs were grown under IDX conditions and/or co-treated with 30 μg/ml of ethanol extracts of Cinnamomi Ramulus and Cinnamomi Cortex (A). After the induction of adipogenesis, the media were replaced every two or three days. On the 12th day in culture, cell culture supernatants were harvested. ELISA was performed to measure the levels of adiponectin accumulated in the supernatants during the 48 h after the last medium exchange. Cytotoxicity of the major chemical components, cinnamaldehdye, cinnamic acid, and eugenol, on hAT-MSCs was determined using 4-3-[4-lodophenyl]-2-4(4-nitrophenyl)-2*H*-5-tetrazolio-1,3-benzene disulfonate (WST, Roche Molecular Biochemical, Indianapolis, IN, USA). The absorbance of the samples (A_{450}) was determined, and absolute optical density expressed as a percentage of the control value (B–E). The effects of cinnamaldehdye, cinnamic acid, and eugenol on adiponectin production in hAT-MSCs during adipogenesis were evaluated (F). Values represent mean ± SD ($n = 3$). $^{*}P \leqslant 0.05$ and $^{**}P \leqslant 0.01$.

cinnamaldehyde (30 μM) and cinnamic acid (100 μM) in the IDX condition significantly increased adiponectin production (by 70% and 46%, respectively), compared with that in the IDX control. In contrast, eugenol did not promote adipogenesis in hAT-MSCs (Fig. 1F). Therefore, cinnamaldehyde and cinnamic acid may contribute to the effect of the *Cinnamomum cassia* methanol extract in promoting adipogenesis in hAT-MSCs.

Previously, we synthesized the 3,4-methylenedioxy cinnamate derivative of kojic acid (**4b**, Seletinoid G, Scheme 1) as a new anti-aging compound.[12] Kojic acid is produced from carbohydrate sources in an aerobic process by a variety of microorganisms.[13] It exerts various biological effects-such as an inhibition of tyrosinase, chelation of metal ions, free radical scavenging, and prevention of photo-damage due to its γ-pyranone structure containing an enolic hydroxyl group.[14] Compound **4b** was designed based on the assumption that the γ-pyranone ring of kojic acid would mimic the carboxylic acid moiety in retinoid structures. Because retinoic acid derivatives have in vivo anti-aging activity in human skin, we intuitively expected that compound **4b** may counteract the functional regression of aged human skin. In human clinical studies, we demonstrated that compound **4b** increased procollagen synthesis and decreased matrix metalloproteinase-1 (MMP-1) in photo-aged human skin in vivo.[12] Because compound **4b** also contains a cinnamate moiety, we evaluated whether, like cinnamaldehyde and cinnamic acid, it could affect adipogenesis in hAT-MSCs. In a preliminary screen, we found 100 μM of **4b** promoted adiponectin production in hAT-MSCs (data not shown). Therefore, to study the structure activity relationship of kojyl cinnamate derivatives of **4b** in promoting adiponectin biosynthetic activity in hAT-MSCs, we synthesized additional cinnamate derivatives (**4a** and **4c**) and modified compounds (**4d**–**4f**).[15] The synthetic pathways are shown in Scheme 1. Cinnamate derivatives (**4a**–**4c**), a benzoate derivative (**4e**), and a hydrocinnamate derivative (**4d**) were synthesized by the condensation of kojyl chloride **2**