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**Polyphenol-rich apple extract inhibits dexamethasone-induced sebaceous lipids
production by regulating SREBP1 expression**

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Abstract

Sebum production and excretion is a primary function of the sebaceous glands, but abnormally increased sebum production is a major cause of acne vulgaris. To identify a new candidate that regulates sebum production, we investigated the possible inhibitory effects of apple polyphenols (APP) purified from unripe apples on primary cultured human sebocytes and in patients with acne vulgaris. Dexamethasone (Dex) increased lipid synthesis and expression of the sterol response element-binding protein 1 (SREBP 1) and its target enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), in the sebocytes. However, APP inhibited Dex-induced lipid production and expression of SREBP-1, ACC, and FAS. APP also inhibited the increase in the expression and activation of glucocorticoid receptor (GR) in the sebocytes. Taken together, these results suggest that APP may be useful to regulate sebum production and may alleviate sebum-involved skin disease, such as acne vulgaris.

Background

Acne is a common skin disorder of the pilosebaceous unit, and multiple factors, such as increased sebum production, androgen metabolism, inflammatory reaction, interaction with neuropeptides, follicular hyperkeratinization, and the action of *Propionibacterium acnes* (*P. acnes*) within the follicle, have been implicated in its pathogenesis (1-4). Among these factors, abnormally increased sebum production is a major contributing event. Sebaceous glands secrete sebum to protect the skin's surface from various environments. However, increased sebum production may disturb the process of follicular keratinization in the pilosebaceous units. (5,6). Glucocorticoids (GCs), steroid hormones, induced sebaceous

lipogenesis by activating glucocorticoid receptor (GR), and dexamethasone (Dex)-induced sebaceous lipid production could be mediated in part through the expression of the transcription factor sterol response element-binding protein 1 (SREBP-1) (7), which directly up-regulates downstream target enzymes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (8, 9). Therefore, ingredient inhibiting lipid synthesis-related gene by regulating GR may alleviate stress-induced abnormal sebum production in acne vulgaris.

In previous studies, polyphenols from apples have been reported to have effects of anti-oxidative stress and anti-inflammation. Apple polyphenols (APP) extracted and purified from unripe apples is composed of procyanidins, catechins, phenol carboxylic acids, and other compounds (S1-S4).

Question addressed

APP has been reported to have beneficial actions on oxidative stress and inflammation. However, the effect of APP on sebaceous lipids production has not yet been examined. In the present study, we investigated the possible inhibitory effects of apple polyphenols (APP) purified from unripe apples on sebaceous lipids synthesis and its biological actions in primary cultured human sebocytes.

Experimental design

Sebaceous glands were isolated from separated hair follicles and transferred to a tissue culture dish. We examined the effects of APP on in Dex-induced lipid production, lipid synthesis-related gene, and GR expression and activation by Oil red O staining, real-time RT-PCR, and Western blotting assay. The materials and methods are described in the supplementary online material (Data S1).

Results

Primary cultured human sebocytes were treated with Dex to induce sebaceous lipid synthesis. We found that Dex induced the sebaceous lipid synthesis beyond the testosterone/linoleic acid combination, but APP inhibited Dex-induced lipid accumulation within the sebocytes (Fig. 1a). Dex also increased both the mRNA and protein levels of SREBP-1, ACC, and FAS. However, APP significantly inhibited the increase in these genes at both mRNA and protein levels in a dose-dependent manner (Fig. 1b-f, S1). These results suggest that APP inhibits the increase in sebaceous lipids production through down-regulating lipid synthesis-related genes in sebocytes. We next investigated the effect of three polyphenols on the expression of SREBP-1 in Dex-treated sebocytes. Catechin, epicatechin, and procyanidin B₂ inhibited the increase in SREBP-1a and SREBP-1c expressions (Fig. S3). These results imply that the main single compounds from apple inhibit abnormally increased lipid synthesis; especially, the procyanidin B₂, the most abundant polyphenols in apples (S2, S5) in APP, may be the major component that regulates the increase in sebaceous lipids production in sebocytes.

To explain the mechanism through which APP inhibits sebaceous lipids synthesis in Dex-treated sebocytes, the expression and phosphorylation of GR were examined by Western blotting. Dex induced the rapid phosphorylation of GR at 1 h and maintained that at a high level for 12 h. Dex also increased the expression of GR in a dose-dependent manner and maintained that high level for 24 h, but the highest level was observed at 12 h (Fig. 2a). Next, sebocytes were pre-treated with APP or RU486 (GR inhibitor) for 18 h and treated with Dex for 1 h. APP inhibited both phosphorylation and expression of GR in a dose-dependent manner (Fig. 2b). These results suggest that APP affects the expression of lipid synthesis-related genes through regulating both the phosphorylation and expression of GR in sebocytes.

Conclusions

As polyphenols from plants, such as fruits and vegetables, have various pharmacological effects, they have been actively studied as potential therapies for human diseases. EGCG, a major polyphenol in green tea, improves acne by inhibiting excessive sebaceous lipids production, inflammatory cytokines, and *P. acnes* overgrowth (S6, S7). Apples are also known as an important source of polyphenols and possess a variety of biological properties (S8, S9). In this study, we evaluated the effect of APP extracted and purified from unripe apples on sebaceous lipids production.

Excessive sebum production is a key factor that induces acne vulgaris. In a prior study, GCs, induced sebaceous lipogenesis by activating GR and Dex-induced sebaceous lipid production mediated in part through the expression of the transcription factor SREBP-1 (7). GCs are regulated in a circadian- and stress-related manner to maintain various functions of metabolism and homeostasis and are known to affect the pathophysiology of the sebaceous glands, causing the development or exacerbation of acne lesions (S10-S12). Dex-treated sebocytes increased SREBP-1 mRNA levels in GR-dependent manner, suggesting that the influence of GCs on sebaceous lipid synthesis may be partially mediated by a GC-induced increase in SREBP-1 (7). The results of our study showed that Dex treatment augmented intracellular lipid synthesis, but APP attenuated the Dex-induced lipid production through suppression of SREBP-1 and its target genes expression levels in sebocytes. In addition APP inhibited both the phosphorylation and expression of GR. These results suggest that APP inhibits the expression of lipid synthesis-related genes through regulating both the phosphorylation and expression of GR in sebocytes.

In this study, we found that APP decreases the Dex-induced expression of SREBP-1 through inhibiting GR, thereby suppressing intracellular lipid production. As procyanidins have a higher anti-inflammation activity (S2) and EGCG also modulates inflammation and *P. acne* overgrowth (S6), more beneficial properties of APP in acne vulgaris could be expected. Therefore, further studies are suggested to evaluate the inhibitory effects of APP on inflammation and *P. acne* activity in acne vulgaris.

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Figure Legends

Figure 1. The effect of APP on lipid synthesis in Dex-treated sebocytes. Sebocytes were treated with 10 μM Dex for 24 h, and 50 $\mu\text{g/ml}$ APP was applied at the same time (a).

Intracellular lipids in sebocytes were detected by microscopy after Oil red O staining.

Magnification, $\times 400$. The effect of APP on SREBP-1, ACC, and FAS protein levels (b) and mRNA levels (c-f) were assessed. Sebocytes were treated with APP (1-50 $\mu\text{g/ml}$) and Dex (10 μM) for 24 h. Values are relative to the control. The results are representative of three independent experiments from different donors and expressed as mean \pm S.D. (% control) of three technical different cultures. # $p < 0.05$, ## $p < 0.01$ indicate a significant difference from the Dex-treated control and ** $p < 0.01$ indicates a significant difference from control. Equal protein loading was confirmed by staining the membrane with antibodies against β -actin.

Figure 2. The effect of APP on GR in Dex-treated sebocytes. The expression and phosphorylation of GR (a) were assessed by Western blotting at different time points after Dex (1 or 10 μM) treatment. The effect of APP on GR (b) was also assessed in the same way.

Sebocytes were pretreated with APP (1-50 $\mu\text{g/ml}$) or RU486 (1 μM) for 18 h and treated with Dex (1 μM) for 1 h. Each blot is representative of three independent experiments from different donors. The results are expressed as mean \pm S.D. (% control) of three technical different cultures. # p <0.05, ## p <0.01 indicate a significant difference from the Dex-treated control and * p <0.05, ** p <0.01 indicates a significant difference from control. Equal protein loading was confirmed by staining the membrane with antibodies against β -actin.

Fig 1.

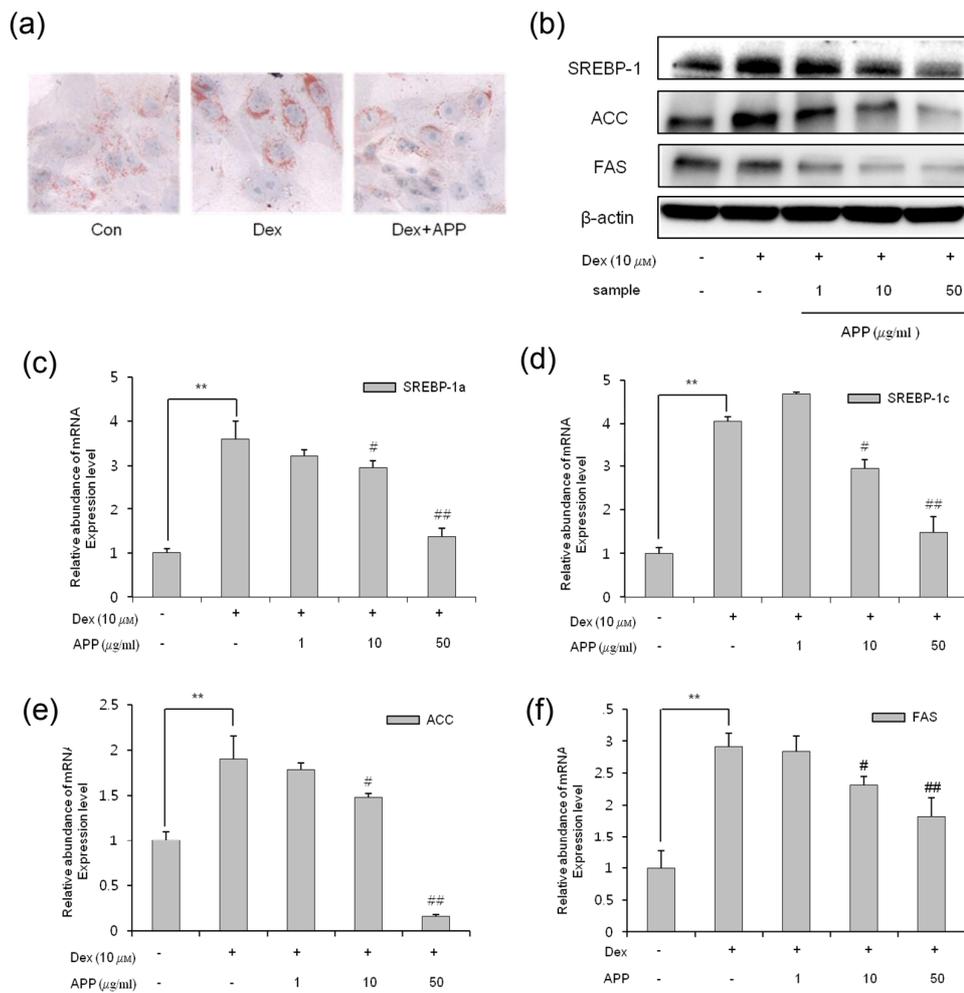


Fig 1.

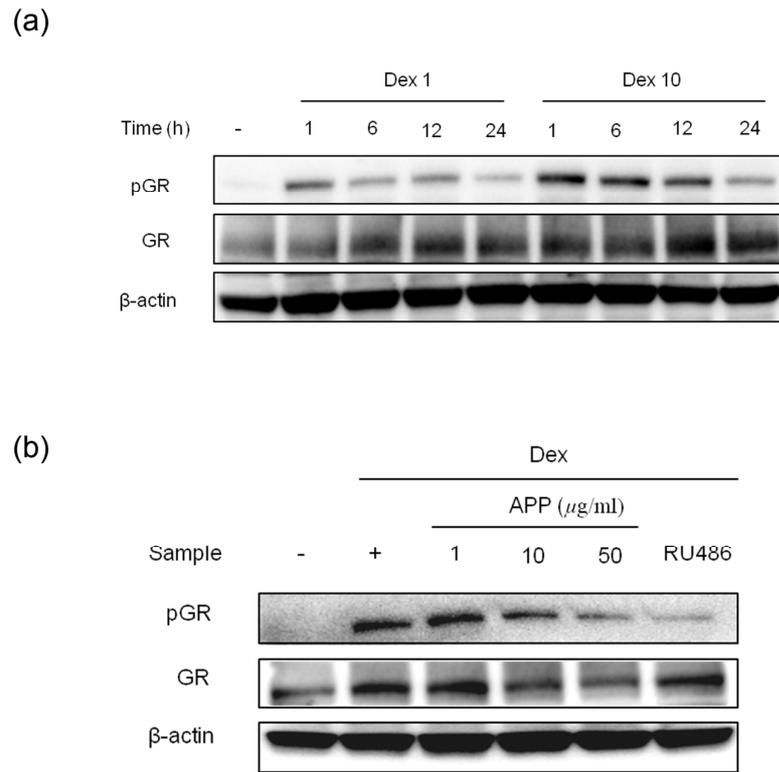


Fig 2.