Sebaceous glands (skin) play an important role in maintaining physiological functions by forming a biological barrier in the skin (Fig. 1). The decrease of sebaceous levels in the skin is thought to depress the barrier functions, and thereafter may be associated with the development of dry skin (xerosis) with a variety of complaints including a rough or scaly surface, and pruritus (Fig. 1). Therefore, a novel aspect to control sebaceous lipogenesis might be beneficial for the prevention of dry skin and seborrheic complaints. In the present study, we investigated the effects of an ethanol extract of Grifola frondosa (Maitake) fruit body (Gripin®) on sebum production in hamsters and humans and compared these effects with those of an ethanol extract of Agaricus blazei murrill (Agaricus) in vivo and in vitro.

**Results**

1. When hamster sebocytes were treated with Gripin®, the intracellular lipid droplet formation including TG, squalene, free-fatty acids, and cholesterol were augmented when compared with the vehicle control treated skin.

2. Gripin® preferentially augmented the synthesis of triacylglycerols (TG), a major sebocyte component, in hamster sebocytes (Figs. 4 and 5). The augmentation of TG production resulted from the increased cellular DGAT activity in hamster sebocytes (Fig. 5, inserted panel).

3. The topical treatment of hamster auricles with 1-4% Gripin® augmented sebocyte accumulation in sebaceous glands (Fig. 6).

4. Another ethanol extract prepared from A. blazei murrill (Agaricus) showed less or no effect on sebaceous lipogenesis in hamsters in vivo and in vitro (Figs. 3, 4, and 6).

5. In most of the volunteers treated with 0.1 and 1% Gripin® cream, the levels of sebaceous components, including squalene, free fatty acids, and cholesterol were increased when compared with the vehicle control treated skin.

**Conclusion**

These results provide novel evidence that Gripin® augments sebaceous lipogenesis in hamsters and humans in vivo and in vitro. Thus, Gripin® is likely to be a unique anti-dry skin agent with lipogenic actions for sebocytes.

**Materials & Methods**

**Treatment**

Female auricles were treated with Gripin® (50-100 mg/ml) or the ethanol extract of A. blazei murrill (Maitake) (100-400 mg/ml) in DMBT12 volunteers with the exposure time of 0-7 days.

**Oil red O staining**

After the treatment of sebocyte with Gripin® or Agaricus, cells were washed with 0.9% NaCl in isotonic phosphate-buffered saline (pH 7.4), and then fixed with a light microscope-furnished with a digital camera. The cells were also counterstained with Mayer's hematoxylin solution.

**Quantitative analysis of sebaceous lipids**

The auricular cells were subjected to an automatic thin-layer chromatography. Lipids, as previously described (1). Otherwise, the auricular cells were used to measure the level of intracellular TG using Oleum TG kit according to the manufacturer’s instructions. The intracellular DGAT activity was determined using Oleum TG Kit (Mitsubishi Chemical, Tokyo, Japan) and 3β-3H-dihydroxy-5β-cholestanol as the substrate described (5, 6).

**In-vivo experiments in hamsters**

Auricles of 3 week-old male golden hamsters were treated topically with 1-4% Gripin® or Agaricus extract in 95% ethanol and 5% glycerol (Maitake, 100 mg/ml) or 0.1% vehicle (5). The data were expressed as mean ± standard deviation. The significance was determined using an ANOVA followed by the Student-Newman-Keuls test (7). The difference was considered statically significant when p < 0.05.

**Table 1**

<table>
<thead>
<tr>
<th>Sebum components</th>
<th>The number of volunteers whose lipid levels in the skin were increased</th>
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<tbody>
<tr>
<td>Squalene</td>
<td>0.1% Gripin®: 6/7, 1% Gripin®: 6/7</td>
</tr>
<tr>
<td>Wax ester</td>
<td>0.1% Gripin®: 4/7, 1% Gripin®: 4/7</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>0.1% Gripin®: 3/7, 1% Gripin®: 3/7</td>
</tr>
<tr>
<td>Free-fatty acids</td>
<td>0.1% Gripin®: 6/7, 1% Gripin®: 6/7</td>
</tr>
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</table>

When 0.1 and 1% Gripin® cream, and vehicle cream were topically treated three times a day for 14 days on the anterolateral regions of seven healthy volunteers (six males and one female, age: 22-41 years), a thin-layer chromatographic analysis was performed to quantify the levels of sebaceous components on the skin surface.

**References**


**Figures**

- Fig. 1 Sebaceous glands in the skin. Left panel, oil red O and hematoxylin staining in the skin of hamster.
- Fig. 2 Ethanol extract of Grifola frondosa (Maitake) fruit body (Gripin®). A, Grifola frondosa (Maitake); B, dry powder of Maitake; C, ethanol extract of Maitake, designated Gripin®.
- Fig. 3 Gripin® augments the formation of lipid droplets in hamster sebocytes. A, control; B, Gripin® (400 μg/ml); and C, the ethanol extract prepared from A. blazei murrill (Agaricus) (400 μg/ml). The cells were stained with oil red O.
- Fig. 4 Gripin® increases the intracellular levels of TG in hamster sebocytes ***, significantly different from untreated cells (C) (p<0.05).
- Fig. 5 Gripin® preferentially augments TG synthesis by increasing DGAT activity in hamster sebocytes. Inverted phase contrast, ** and *** significantly different from untreated controls (Control) (p<0.05 and 0.01, respectively). ChoE, cholesterol ester; WE, wax ester; TG, triacylglycerols; FFA, free-fatty acids; and Cho, cholesterol.