Visualizing the Pharmacology of a Diglyceride-acyltransferase (DGAT) Inhibitor in Skin after Oral Dosing by MALDI Imaging Mass Spectrometry: from Pre-clinical to Clinical

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Introduction

Diacylglycerol acyltransferase (DGAT) catalyzes the final step of triglyceride (TGs) synthesis by combining a fatty acyl-CoA with a diacylglycerol (DG). Acne vulgaris is a chronic skin disease associated with excessive production and secretion of sebum, which contains TGs as a major component. In the sebaceous glands, GlassonSmithKline has developed a DGAT-1 inhibitor, targeting DGAT inhibition as a treatment for acne vulgaris by reducing the TG level in the skin. The compound was orally administered to dogs in a pre-clinical study and to two cohorts of humans in a phase-I clinical study. The skin biopsies from both preclinical and clinical studies were submitted for MALDI IMS analysis to assess the changes associated with treatment following oral administration.

Methods

All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee at GSK or by the ethical review process at the institution where the work was performed. The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents.

• Frozen skin biopsies from dogs and humans were sectioned at a thickness of 10 µm. The matrix (DHB) were applied to sections using a Tm sprayer (HTX Technologies, Canttore, NC, USA) followed by MALDI Imaging MS experiments at 50 µm lateral resolution.
• All MALDI IMS experiments were performed using a Solarix T7 Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA). The MALDI IMS data were assessed through multivariate analysis provided by SCiLS Lab 2017a (Bruker Daltonics, Billerica, MA).
• PCA was performed as a supervised statistical test to determine whether GSK-A treatment induced endogenous changes in the skin.
• ROC analysis was applied to find the biomarkers between the control and the treated groups.
• Additional sections from dog biopsies were collected for LC-MS analysis. The data were processed for lipid identification by LipidStar v1.0.2 (Molecular Discovery Ltd., Peru, Italy).
• Part of the dog skin biopsies were submitted for TaqMan gene assay to quantify the expression of 18 genes directly related to TG metabolism.

Results

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Dog Skin Lipid Profile Changes Induced by Administration of a DGAT-1 inhibitor GSK-A

• Oral administration of GSK-A induced changes of certain lipids, including TGs, in dog skin, which were identified by multivariate statistical analysis of both MALDI IMS data and LC-MS data.
• The structure identification of the TGs were based on the accurate mass detected by MALDI IMS and the 1 mg/kg/day-treated group.
• Table 1 is the summary of the putative DGs detected to skin samples of dogs treated with different doses of GSK-A—groups can be separated based on the doses.

Table 1. TGs Identified Decreased in Dog Skin after treatment

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<tr>
<th>ID</th>
<th>MW</th>
<th>MALDI IMS Detection</th>
<th>LC-MS Detection</th>
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<td>0.05 mg/kg/day</td>
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<td>0.1 mg/kg/day</td>
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<td>1 mg/kg/day</td>
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- The decrease of the TGs were localized to the epidermis and upper dermis in dog skin following treatment.
- Figure 2 shows the representative distribution of one of the identified TGs, TG (50:3), on dog skin sections from the control and the 1 mg/kg/day-treated groups.
- Figure 2A shows the distribution of TG (50:3) C57H104O6 884.7833 [M+Na]+ 902.8171 [M+NH4]+.
- Figure 2B shows the distribution of TG (50:3) C55H100O6 856.7520 [M+Na]+ 874.7858 [M+NH4]+.
- Figure 2C: H&E staining with epidermis towards the top of the image.
- Figure 3: TG intensities on the whole section, upper dermis and lower dermis of dog skin detected by MALDI IMS.
- Table 2 is the summary of the putative DGs detected to skin samples of dogs treated with different doses of GSK-A and clinical studies, and facilitate understanding pathways associated with the target.

Table 2. Putative DGs Detected to Increase in the Sebaceous Glands of Human Skin after GSK-A treatment

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- DGs changes in dog skin samples after DGAT-1 inhibition by GSK-A treatment were not detected.
- TaqMan assay indicated the down-regulation of three genes involved in hydrolysis of DGs back to DGs and one gene involved in synthesis of DGs from monoacylglycerol after GSK-A treatment.
- Figure 4 shows the pathways related to TGs metabolism and genes detected to be downregulated after GSK-A treatment.
- Figure 5 shows the fold changes of the down-regulated genes in the GSK-A treated skin samples compared to the control.