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

Fang Xie, Reid Groseclose, Stephen Castellino

Bioimaging, GlaxoSmithKline, Collegeville, PA, United States

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. GlaxoSmithKline 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 either 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, Carrboro, NC, USA) followed by MALDI imaging MS experiments at 50 µm lateral resolution.
- All MALDI IMS experiments were performed using a Solarix 7T 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 an unsupervised statistical test to determine whether GSK-A treatment induced endogenous changes in the skin*
  - ❖ *ROC analysis was applied to find the discriminants 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 LipoStar v1.0.2 (Molecular Discovery Ltd., Perugia, 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.

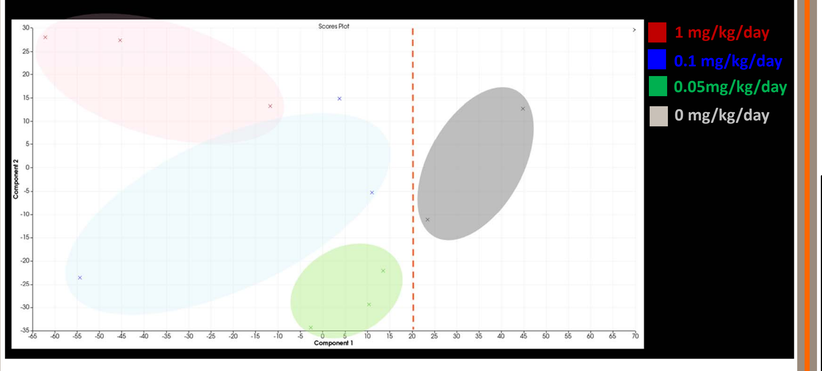
Results

❖ 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 fragments detected by LC-MS/MS

➢ *Figure 1 is the PCA analysis of MALDI IMS data acquired from skin samples of dogs treated with different doses of GSK-A – groups can be separated based on the doses*

Figure 1. PCA Analysis of Dog Skin Treated with GSK-A



➢ *Table 1 is the summary of decreased TGs identified in dog skin after treatment*

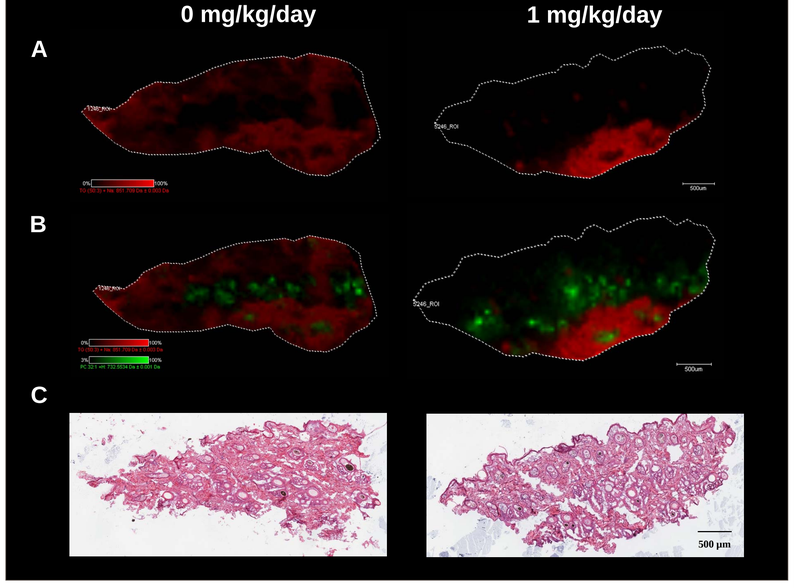
Table 1. TGs Identified to Decrease in Dog Skin

ID	Formula	MW	MALDI IMS Detection		LC-MS Detection	
			m/z	Ion Form	m/z	Ion Form
TG (50:5)	C53H92O6	824.6894	825.6967	$[M+H]^+$	842.7233	$[M+NH_4]^+$
TG (50:4)	C53H94O6	826.705	849.6942	$[M+Na]^+$	844.7388	$[M+NH_4]^+$
TG (50:3)	C53H96O6	828.7207	851.7099	$[M+Na]^+$	846.7545	$[M+NH_4]^+$
TG (52:4)	C55H98O6	854.7363	877.7255	$[M+Na]^+$	872.7701	$[M+NH_4]^+$
TG (52:3)	C55H100O6	856.752	879.7412	$[M+Na]^+$	874.7858	$[M+NH_4]^+$
TG (52:2)	C55H102O6	858.7676	881.7568	$[M+Na]^+$	876.7989	$[M+NH_4]^+$
TG (54:4)	C57H102O6	882.7676	905.7568	$[M+Na]^+$	900.8014	$[M+NH_4]^+$
TG (54:3)	C57H104O6	884.7833	907.7725	$[M+Na]^+$	902.8171	$[M+NH_4]^+$

- 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.*

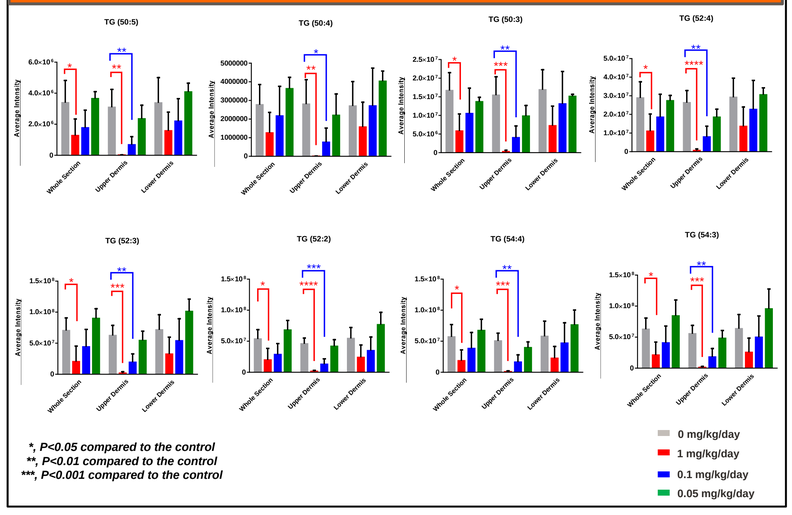
Figure 2. Dog Skin Images with Oral Dosing of GSK-A



- o *Figure 2A shows the distribution of TG (50:3)*
- o *Figure 2B shows the distribution of TG (50:3) overlaid with a sebaceous gland marker PC (32:1)*
- o *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.*

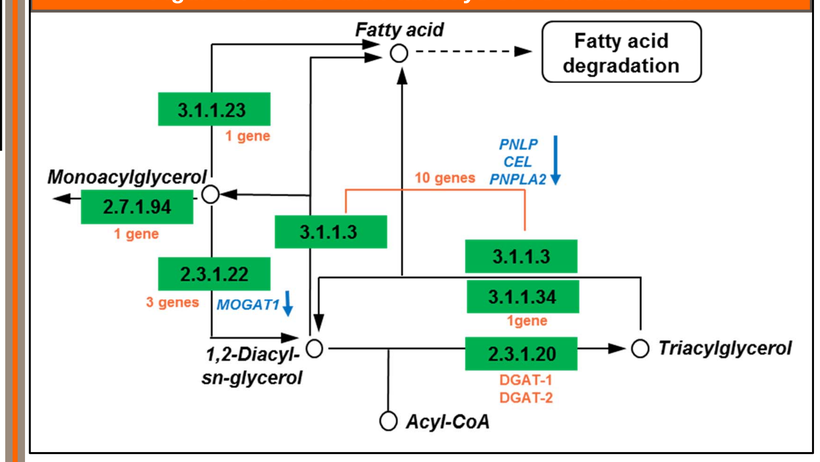
Figure 3. Intensities of the Identified TGs in Dog Skin Sections



- 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 TGs back to DGs and one gene involved in synthesis of DGs from monoacylglycerols after GSK-A treatment

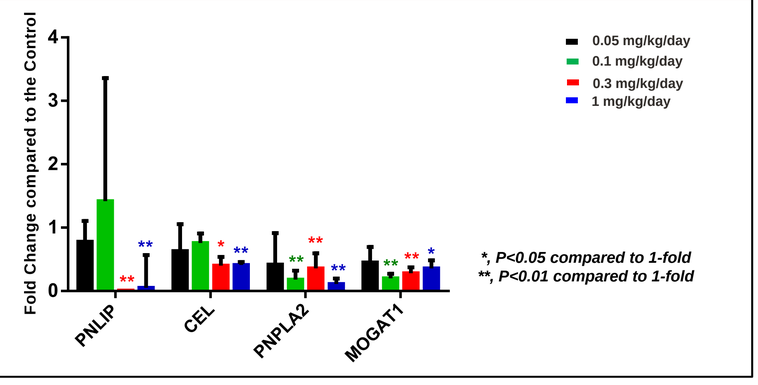
➢ *Figure 4 shows the pathways related to TGs metabolism and genes detected to be downregulated after GSK-A treatment*

Figure 4. Genes and Pathways of TG Metabolism



➢ *Figure 5 shows the fold changes of the down-regulated genes in the GSK-A treated skin samples compared to the control*

Figure 5. Summary of the targeted gene fold changes



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

- Multivariate statistical analysis indicated that GSK-A treatment induced increase of several endogenous ions in the sebaceous gland regions of human skin, which are consistent with DGs based on the accurate mass.

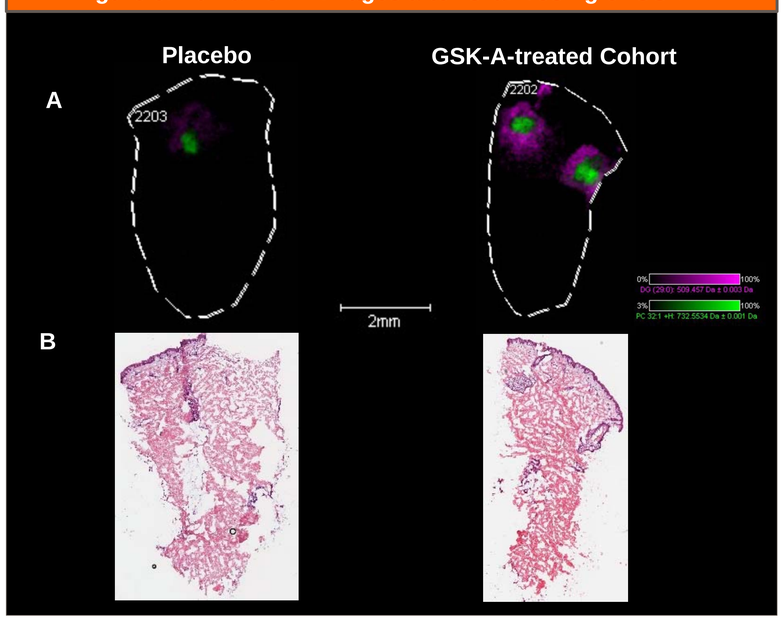
➢ *Table 2 is the summary of the putative DGs detected to increase in the sebaceous glands of human skin after GSK-A treatment*

Table 2. Putative DGs Detected to Increase in Human Skin

ID	Formula	MW	MALDI IMS Detection	
			m/z	Ion Form
DG (29:0)	C32H62O5	526.4597	509.4591	$[M+H-H_2O]^+$
DG (31:1)	C34H64O5	552.4754	535.4747	$[M+H-H_2O]^+$
DG (31:0)	C34H66O5	554.4910	537.4898	$[M+H-H_2O]^+$
DG (33:1)	C36H68O5	580.5067	563.5044	$[M+H-H_2O]^+$
DG (33:0)	C36H70O5	582.5223	565.5222	$[M+H-H_2O]^+$

➢ *Figure 6 shows the representative distribution of one of the DGs, DG (29:0), on human skin section after GSK-A treatment*

Figure 6. Human Skin Images after Oral Dosing of GSK-A



- o *Figure 6A shows the distribution of DG (29:0) overlaid with a sebaceous gland marker PC (32:1)*
- o *Figure 6B is the H&E staining of sections serial to sections shown in 6A*

CONCLUSIONS

- MALDI IMS along with multivariate statistics can identify target-associated pharmacodynamic changes induced by drug treatment in preclinical and clinical studies, and facilitate understanding target-associated pathways and physiology
- ❖ Inhibition of DGAT by GSK-A treatment induced differential changes of skin lipid profiles in the preclinical and clinical studies
- ❖ The differences between species to target inhibition could result in differential changes in the pathways associated with the target

Acknowledgements

We would like to thank Drs. Sara Tortorella and Gabriele Cruciani for assistance with lipid identification. We thank Dr. Deidre Delmas for TaqMan gene analysis.