MSACL, 9.–11. September 2018, Salzburg, Österreich

Determination of eicosanoids by UHPLC-MS/MS in diluted interstitial fluid sampled with open flow microperfusion

Anita Eberl[1], Cornelia Pipper[1,2], Natalie Bordag[2], Bernadette Reiter[1], Kyriakos Economides[3], Peter Florian[4,] Thomas Birngruber[1], Frank Sinner[1], Manfred Bodenlenz[1]

CONTACT

1

JOANNEUM RESEARCH
Forschungsgesellschaft mbH

HEALTH
Institute for Biomedicine and
Health Sciences

Graz, Austria

Anita Eberl

Phone: +43 316 876-4209

anita.eberl@joanneum.at
www.joanneum.at/health

2

CBmed
Center for Biomarker
Research in Medicine

Stiftingtalstrasse 5
8010 Graz, Austria

3

Sanofi, Immunology &
Inflammation Research TA,
Type 2 Inflammation & Fibrosis,

01701 Framingham, MA, USA

4

Sanofi, Immunology &
Inflammation Research TA, Type
1/17 Immunology and Arthritis
Cluster,

Industriepark Höchst,
65926 Frankfurt am Main, Germany

Introduction

Eicosanoids play key roles in inflammatory skin diseases such as psoriasis. Eicosanoids are released close to the source of inflammation where they elicit local, pleiotropic effects and dysregulations. Monitoring inflammatory mediators directly in skin lesions could provide new insights and new therapeutic possibilities.

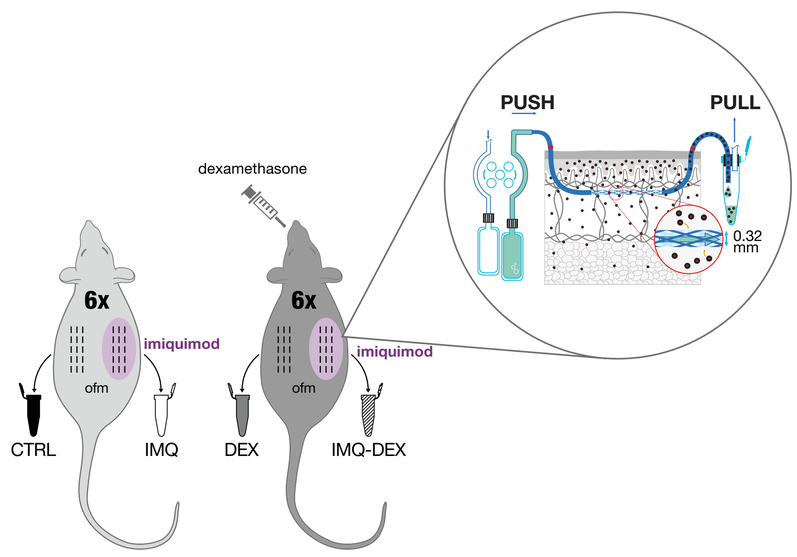

Figure 1: dOFM study design. *All rats were treated with topical imiquimod on one side of the back to induce local inflammation. Six of the twelve rats additionally received systemic, oral dexamethasone (DEX) treatment. From each side samples were collected with three dOFM probes.*

This study describes the analysis of dermal interstitial fluid samples obtained with dermal open flow microperfusion (dOFM) in a rat model of skin inflammation. An SPE-UHPLC-MS/MS method to quantify eleven eicosanoids (TXB_2, PGE_2, PGD_2, $PGF_{2\alpha}$ LTB_4, 15-HETE, 12-HETE, 5-HETE, 12-HEPE, 13-HODE, 17-HDHA) was developed for reliable and precise analysis of small volume samples.

The right side of the figure shows a schematic representation of the dOFM sampling used, demonstrating the collection of diluted interstitial fluid (ISF) directly from skin [1].

Methods

Chromatographic conditions:

- Sample preparation by SPE
- Acquity UPLC® (Waters) BEH C18 (2.1x150 mm; 1,7 µm article column)
- Mobile phase A: water:ACN:FA (63:37:0,02) (v:v:v)
- Mobile Phase B ACN:2propanol (50:50) (v:v); gradient elution

Mass Spectrometric detection:

- Agilent Triple Quad (6495)
- Negative ionization mode
- Internal standardisation
- Dynamic multiple reaction monitoring (dMRM)

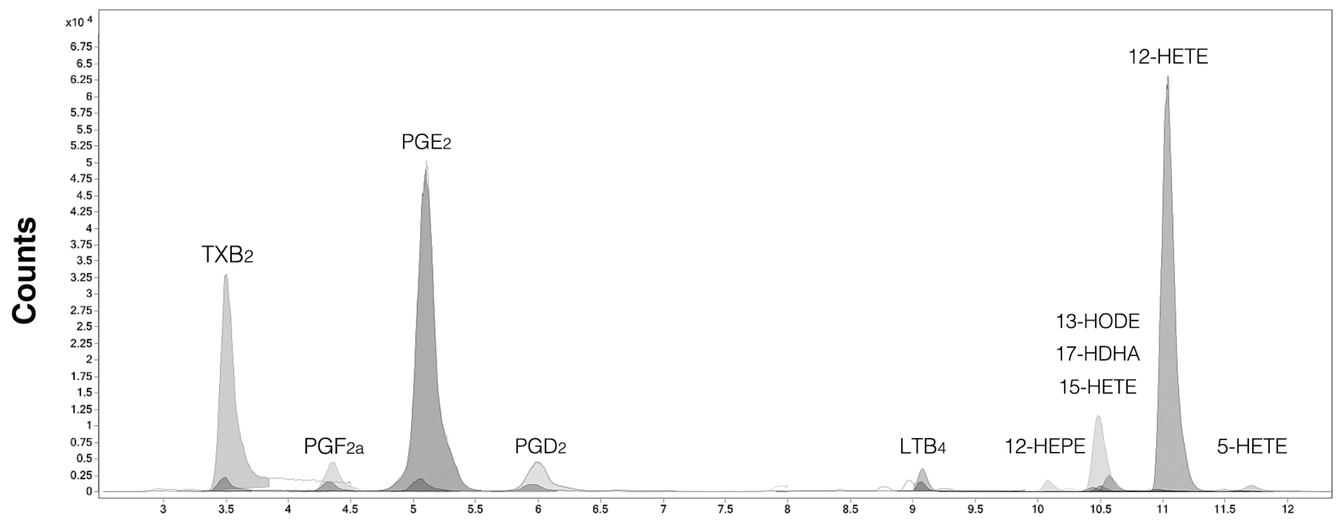

Figure 2: UHPLC-dMRM chromatogram *of the targeted eleven eicosanoids and the corresponding internal standards*

Results

The LC-MS method achieved a median intra-day precision of ~5% and inter-day of ~8%. All calibration curves showed excellent linearity between 0.01 ng/ml to 50 ng/ml ($R^2 > 0.980$).

Eicosanoids were significantly increased in the inflamed skin sites that were treated with imiquimod compared to the untreated control sites. Oral treatment with an anti-inflammatory glucocorticoid decreased eicosanoid concentrations.

Conclusion

The established method for quantification of eicosanoids was sensitive, reproducible, precise, robust and well applicable to rat dermal interstitial fluid samples. A combination of tissue-specific sampling with LCMS analytics was well suited to analyze small sample volumes from minimally invasive sampling methods such as OFM or microdialysis to study local inflammation and the effect of treatments in skin diseases.

References

[1] M. Bodenlenz, B. Aigner, C. Dragatin, L. Liebenberger, S. Zahiragic, C. Höfferer, et al., Clinical applicability of dOFM devices for dermal sampling., Skin Res. Technol. 19 (2013) 474–483. doi:10.1111/srt.12071