High Temporal And Spatial Resolution Open Flow Microperfusion

To Identify Neurological Disease Biomarkers In Rats And Inflammation Biomarkers In Humans
I. Introduction
   a. PD at target tissue
   b. Microdialysis and Open Flow Microperfusion
   c. Biomarker in interstitial tissue fluid

II. Cerebral Open Flow Microperfusion
   a. Introduction
   b. Strengths and weaknesses
   c. Case Study: Cytokine response in rat brain after systemic LPS stimulus

III. Dermal Open Flow Microperfusion
   a. Case Study: Secukinumab®
   b. Pilot Study: Disease specific biomarker in psoriasis

IV. HEALTH – the scientific “one-stop-shop”
PD at target tissue

Blood is an easy to reach compartment, but sometimes you need to “consult” your target tissue!
Continuous Sampling in Target Tissue

**Working principle**

Interstitial fluid

OFM / MD probe: perfusate in direct contact with ISF at constant flow

c=0

Fat layer in skin

Interstitial fluid

c≠0
### OFM and MD application range

<table>
<thead>
<tr>
<th>Substance / Drugs ...</th>
<th>MD</th>
<th>OFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>... small</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>... hydrophilic(small)</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>... larger + large</td>
<td><strong>YES &amp; NO</strong></td>
<td>YES</td>
</tr>
<tr>
<td>... lipophilic (super lipophilic)</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>... protein-bound</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>... (nano)carrier / cells</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

**OFM:** PK/PD of ANY substance independent of size and lipophilicity

**MD:** PK/PD of small and hydrophilic substances
OFM samples represent **diluted but unfiltered** interstitial fluid.

CE certified for clinical use in **dermis** and **adipose tissue**.
Biomarker in Interstitial Tissue Fluid

- Human cytokine multiplexing
- ~ 10 compounds in parallel
- OFM samples, plasma/serum

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<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>A2M</td>
<td>IFN-β</td>
<td>IL-16</td>
<td>MIP-1β</td>
</tr>
<tr>
<td>Albumin</td>
<td>IFN-γ</td>
<td>IL-17A</td>
<td>NGAL/LCN2</td>
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<tr>
<td>Aurora A</td>
<td>IL-1α</td>
<td>IL-17B</td>
<td>Osteopontin</td>
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<tr>
<td>BAD</td>
<td>IL-1β</td>
<td>IL-17D</td>
<td>p38</td>
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<tr>
<td>c-Jun</td>
<td>IL-2</td>
<td>IL-18</td>
<td>P-Selectin</td>
</tr>
<tr>
<td>CRP</td>
<td>IL-4</td>
<td>IP-10</td>
<td>SAA</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>IL-5</td>
<td>JNK</td>
<td>STAT3</td>
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<tr>
<td>Eotaxin-3</td>
<td>IL-6</td>
<td>KIM-1</td>
<td>STAT4</td>
</tr>
<tr>
<td>ERK-1/2</td>
<td>IL-7</td>
<td>LBP</td>
<td>STAT5a/b</td>
</tr>
<tr>
<td>E-Selectin</td>
<td>IL-8</td>
<td>MAPKAPK2</td>
<td>TARC</td>
</tr>
<tr>
<td>FIT-1/VEGFR1</td>
<td>IL-8 (HA)</td>
<td>MCP-1</td>
<td>TIMP-1</td>
</tr>
<tr>
<td>Fractalkine</td>
<td>IL-10</td>
<td>MCP-4</td>
<td>TNF-α</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>IL-12/IL-23p40</td>
<td>MDC</td>
<td>TNF-β</td>
</tr>
<tr>
<td>HSP27</td>
<td>IL-12p70</td>
<td>MEK 1/2</td>
<td>VCAM-1</td>
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<tr>
<td>I-309</td>
<td>IL-12 Total</td>
<td>MEK2</td>
<td>VEGF-A</td>
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<tr>
<td>ICAM-1</td>
<td>IL-13</td>
<td>MIF</td>
<td></td>
</tr>
<tr>
<td>IFN-α2a</td>
<td>IL-15</td>
<td>MIP-1α</td>
<td></td>
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</table>
Biomarker in Interstitial Tissue Fluid

*biomarker screening*

- The OLINK technique uses pairs of antibodies equipped with DNA reporter molecules which are amplified and quantified by high throughput real-time PCR.
- Different panels available, Inflammation: 92 cytokines
- Only 1 µL sample volume needed

**Proximity Extension Assay (PEA)**

- Incubation: o/n
- Extension: 2 hrs
- Detection: 4.5 hrs

http://www.olink.com/
Biomarker in Interstitial Tissue Fluid

*biomarker screening by HPLC-MS/MS*

**Targeted Metabolomics:**
set of known metabolites

**Untargeted Metabolomics:**
comparison of profiles

10 metabolites from 40 µl
Open Flow Microperfusion applications

Neurology
- neurodegenerative diseases
- brain
- BBB transport characterization

Dermatology
- psoriasis, acne, cancer,…
- skin

Endocrinology
- endocrine diseases, diabetes,…
- muscle and fat-tissue

to determine PK/PD of drugs and bioequivalence
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IV. HEALTH – the scientific “one-stop-shop”
cOFM

cerebral open flow microperfusion

A long term interface to brain tissue with intact blood-brain barrier
The Blood-Brain Barrier (BBB)
Blood-Brain Barrier Transport

Importance

Target Tissue Access facilitates:

- Disease biomarker search and validation
- Drug effect evaluation and drug development

Abbott JN. 2013; Blood-brain barrier structure and function and the challenges for CNS drug delivery. The Innovative Medicines Initiative (IMI) Research Agenda 2008;
cOFM probe implantation

frontal lobe

brain tissue
cOFM
working principle II

cOFM probe for rodents
OD probe: 0.5 mm
Material: PEEK, PTFE
Probe body: 5 mm
Weight: ~90 mg

cOFM in mouse

cOFM and MD in rat
cOFM accessed brain areas in rodents

Frontal lobe  Hippocampus

- Hypothalamus
- Striatum
- Multiple probe application is feasible

cOFM sampling can be modified to access various brain areas and to be used in mouse models.
cOFM

**CSF sampling**

- Sampling in cisterna magna
- Sampling in the lateral ventricle
- 25 µl/h (rat)
- Compare CSF to ISF

*ISF and CSF parallel sampling in rats and mice*
tissue response to probe implantation

Trauma layer surrounding microdialysis membrane
(Benveniste and Diemer 1987)

No trauma layer surrounding cOFM probe after 2 weeks

No continuous glial scar 30 days after cOFM probe implantation

Birngruber et al. 2014 PLoS ONE
cOFM tissue response to probe implantation

Quantification of astrocytes 15 days after cOFM implantation

Quantification of microglia 15 days after cOFM implantation
cOFM

BBB healing after implantation trauma

BBB permeability marker - Evans Blue (EB)

Birngruber et al. 2013 Clin Exp Pharmacol Physiol
cOFM

BBB monitoring during sampling

Marker: sodium fluorescein - Naf (376 Da)

Birngruber et al. 2013 Clin Exp Pharmacol Physiol
Background and Objectives
- Investigate the effect of systemic LPS to brain response

Set-up
- Treatment and control group (n=6 each)
- cOFM probe implantation in frontal cortex on day 0
- LPS or saline injection combined with Naf on day 15
- cOFM sampling for 6 hours after LPS or saline injection

Read-out
- TNF-alpha, IL-6 in serum and cerebral ISF ➔ pro-inflammatory response
- IL-10 in serum and cerebral ISF ➔ anti-inflammatory response
- Naf in cerebral ISF ➔ BBB intactness
case study: response to LPS injection

- TNF-alpha concentrations in cISF and serum increased rapidly
- TNF-alpha in brain rests elevated
- TNF-alpha in cISF and serum is higher than in control group
- Delayed IL-6 peak in cISF and serum (4–6 h) after LPS injection
- IL-6 in cISF and serum is higher than in control group
case study: response to LPS injection

Anti-inflammatory response

- Delayed IL-10 peak in cISF (>6 hours) and serum (4–6 h) after LPS injection
- IL-10 in cISF and serum is higher than in control group

Conclusion

Peripheral LPS administration induced severe BBB dysfunction

→ increased production of pro-inflammatory (TNF-alpha and IL-6) and anti-inflammatory (IL-10) cytokines in brain interstitial fluid

→ TNF-alpha concentrations in brain extracellular fluid and serum increased rapidly, whereas brain IL-6 and IL-10 production lagged behind approximately 1 h.
Biomarker research

- TNF-alpha, IL-8 and IL-10 response in cISF and CSF to the “double hit” - priming with lipopolysaccharide (LPS) and activation of the P2X7 receptor with BzATP
- Metabolome profile in CSF and ISF in a focal EAE rat model

Drug research

- Quantification of an antibody in cISF after systemic administration
- Drug concentration in cISF after systemic administration of Amitryptilin and other drugs
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IV. HEALTH – the scientific “one-stop-shop”
This study was an exploratory, open-label, 2-part, single-center study (NCT01539213)

- A single 300-mg s.c. dose of secukinumab was administered on Day 1 (after baseline samples were obtained) to 8 healthy volunteers (Part 1) and 8 plaque psoriasis patients (Part 2)
  - Protein levels of IL-17a cytokine subtypes and β-defensin-2 in dermal Interstitial Fluid (ISF) were sampled using dOFM at baseline and on Days 8 and 15
  - Sinistrin, a polysaccharide not metabolized in the human body, served as a reference for dOFM stability and quantification of dermal ISF concentrations of IL-17 cytokines and β-defensin-2
  - Secukinumab was quantified in dermis
dOFM

target validation: IL-17a

Baseline IL-17A in Skin of Psoriasis Subjects and Healthy Volunteers (Mean + SE)

Baseline IL-17F in Skin of Psoriasis Subjects (Mean + SE)

*One value >67 pg/mL for lesional skin not shown in figure for clarity.

***P< 0.0001, lesional vs. non-lesional skin and lesional vs. healthy skin.

Calculated absolute values take into account mean relative recovery rate of the reference molecule (sinistrin).

The data below LLOQ were imputed by ½ LLOQ.

IL-17A, but not IL-17F, is significantly higher in psoriatic lesional skin compared with non-lesional skin or skin of healthy volunteers.
Postulated mode of action - down stream IL17a marker

β-Defensin-2 protein levels are elevated in psoriasis lesional skin and serum and decrease rapidly in response to secukinumab treatment.
Protein level of mediators for keratinocyte proliferation and angiogenesis and keratinocyte mobility

Protein levels of amphiregulin and epiregulin were reduced within 7 days → driver of autocrine keratinocyte proliferation,

Expression of gelatinase B (MMP-9) protein was reduced within 15 days → implicated in angiogenesis and tissue destruction
**dOFM**

*dermal secukinumab levels to neutralize IL-17a*

**Absolute quantification** of secukinumab in the dermis of healthy volunteers

<table>
<thead>
<tr>
<th>Serum and Dermal Secukinumab Levels (μg/mL, mean ± SD)</th>
<th>Healthy Volunteers (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum</strong></td>
<td><strong>Dermal ISF</strong>&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Day 8</strong></td>
<td><strong>Day 8</strong></td>
</tr>
<tr>
<td>36.1 ± 10.5</td>
<td>7.76 ± 1.30</td>
</tr>
<tr>
<td>35.0 ± 10.5</td>
<td>8.02 ± 3.23</td>
</tr>
</tbody>
</table>

Dermal ISF concentrations ~22% (Day 8) and ~23% (Day 15) of serum
Dermal concentration by OFM, blister fluid and in biopsies are comparable
**Absolute quantification of Secukinumab in dermis in psoriatic patients**

<table>
<thead>
<tr>
<th>Serum and Dermal Secukinumab Levels (μg/mL, mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Psoriatic Subjects (n = 8)</strong></td>
</tr>
<tr>
<td><strong>Serum</strong></td>
</tr>
<tr>
<td>Day 8</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>21.1 ± 4.3</td>
</tr>
<tr>
<td><strong>Dermal ISF^{a,b}</strong></td>
</tr>
<tr>
<td>Day 8</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>6.76 ± 2.68</td>
</tr>
<tr>
<td>5.65 ± 1.80</td>
</tr>
</tbody>
</table>

Dermal ISF concentrations are 28-39% of serum concentration
Dermal ISF concentrations on Day 8 and 15 are similar
IL-17a is elevated in psoriatic plaque

β-Defensin-2 is reduced by secukinumab

Key mediators of keratinocyte proliferation, and MMP-9, implicated in angiogenesis and keratinocyte mobility, were also reduced

Secukinumab concentration in skin is sufficient to neutralize IL-17a in psoriatic skin

dOFM is a useful tool to investigate PK and PD aspect in disease research and drug development.
dOFM

*pilot study: cytokine screening*

- one psoriatic patient (6 samples)
- one healthy subject (4 samples)
- Cytokine profiling using Olink® inflammation panel
- 57 cytokines were above LOD (out of 92)
- 4 cytokines were significantly different

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dOFM is an useful tool for a holistic biomarker approach by cytokine analysis or characterization of immune-competent cells using FACS analysis
Close Cooperation

Joanneum Research - Medical University of Graz

58 general wards (~1400 beds)
10 intensive care units (~140 beds)
16 outpatient clinics
80,000 patients/year
10,000 patients/year
500,000 patients/year
One-Stop-Shop
for tissue specific PK and PD

aOFM
adipose-tissue-OFM

Ex vivo
preclinical

bOFM
cerebral-OFM

In vivo
clinical

cOFM
dermal-OFM

Bioanalytics

Statistics

Reporting

Study design

Ethics

Ex vivo
preclinical

In vivo
clinical

EN ISO 9001

EN ISO 13485:2003

GLP

Data management

statistic

GCP
Preclinical facilities

mice, rats, rabbits, pigs, sheep
Clinical Facilities

**phase 1-2**

- Fully equipped clinical trial center with 12 beds
- Study performance according to GCP
- Located at the Medical University of Graz
HEALTH

bioanalytics and metabolomics

- Located at the Center of Knowledge and Technology Transfer in Medicine (“ZWT”) in Graz
- High end bioanalytical lab facility of the HEALTH Institute of Biomedicine and Health Sciences
Thank you for your attention

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