## Impact of local inflammation and blood-brain barrier (BBB) impairment: comparison of In vivo microdialysis and cerebral open flow microperfusion (cOFM) methods for the detection of cytokines

## Conclusions

436.18

- COFM method is applicable for measurements of large molecules in brain ISF and CSF. IL-1 $\beta$ , TNF $\alpha$  and IL-6 were quantified using MSD platform assay;
- In vivo microdialysis in freely moving animals can be used to study CNS cytokines, including IL-1 $\beta$ , IL-1RA, TNF $\alpha$  and IL-6
- K Measurements of TNFα in response to stimulation differ between cOFM and *in vivo* microdialysis in timing, magnitude and pattern;
- \* The difference is most likely due to the local tissue reaction and permeability of BBB thus affecting neuroinflammatory response as well as to filtering effects and adsorption on the microdialysis membrane.

## Introduction

Neuroinflammation is a main component of several neurological disorders and is primarily mediated by microglia, the myeloid cell population of the CNS. IL- $1\beta$  is a central mediator of neuroinflammation, which is released from activated microglia. This process involves a priming step to induce pro-IL-1 $\beta$  and a triggering event leading to the processing and release of mature IL-1 $\beta$ . This "double hit" paradigm is experimentally mimicked by priming with lipopolysaccharide (LPS) and activation of the P2X7 receptor with an agonist (2'(3')-O-(4-benzoylbenzoyl)adenosine-5'-triphosphate BzATP such tri(triethylammonium). We have compared microdialysis to a novel in vivo technique - cOFM which was used to continuously sample interstitial fluid (ISF) in the frontal cortex and cerebrospinal fluid (CSF) in the ventricular space for cytokine measurements in response to a "double hit

### **Experimental Design**

**Animals and surgery.** For microdialysis surgeries the animals were placed in a stereotaxic frame, under isoflurane anesthesia. Guide cannulas (BrainLink, Groningen, The Netherlands, brainlink.nl) were implanted aiming into the PFC and an additional for ICV injections was implanted into the lateral cerebral ventricle. Both implantations were completed in one surgery. For cOFM probe implantation, the rats were anaesthetised with a combination of Fentanyl (5 µg/kg), Midazolam (2 mg/kg) and Medetomidin (0.15 mg/kg) and 1 mm holes were drilled in the skull. The cOFM probe was inserted slowly into the left neocortex and into the cisterna magna. cOFM probes were fixed to the skull bone with anchor screws and dental cement. After surgery, the animals received subcutaneous injection of Cefotaxim 5 mg/100 and Rimadyl 0.5 mg in 0.5 ml normal saline (carprofen 50 mg/ml) and continued for 2 more days after surgery. The surgical procedure was completed within 1 hour after initial induction with Isoflurane for each rat. Following surgery, rats were individually housed in specially designed cages under same conditions. **"Double hit" paradigm.** The LPS (3 µg/5µL) and BzATP (87 µg/5µL) were

given icv. Samples were collected every 60 minutes for 8 hours. During microdialysis sampling and cOFM, the conscious, unrestrained animals were housed in an conscious animal containment system. All samples were kept in -80°C until cytokine analyses using MesoScale Discovery (MSD) platform ELISA assay.

Dekun Song, Thomas Birngruber, Gamini Chandrasena, Frank Sinner and Gennady N. Smagin Lundbeck Research USA, Paramus, NJ, USA and Inst. for Biomedicine and Health Science, Joanneum Research, Graz, Austria



In vivo cOFM and CSF sampling coupled with MSD assay allowed measurements of IL-1B, IL-6 and TNFa in the brain ISF and CSF



SFN Annual Meeting, CHICAGO, IL, USA Monday, October 19, 2015, 1:00 PM - 5:00 PM



Push-pull microdialysis. One week after surgery, microdialysis probes were inserted via the guide The animal was then cannula. placed in a plastic observation cage and connected to a 2channel swivel. The input of the dialysis probe was connected to a syringe pump, which delivered an artificial cerebrospinal fluid (CSF) with 0.15% HSA at a rate of 0.7 µl/min overnight. The output from the swivel was attached to a peristaltic and pump refrigerated fraction collector.





E-mail GNS@LUNDBECK.COM for a PDF copy





# JOANNEUM RESEARCH

## Rat Microdialysis



*In vivo* microdialysis method coupled with MSD assay allowed measurements of IL-1 $\beta$ , IL-6 and TNF $\alpha$  in the brain ISF

