# CHARACTERIZATION OF A NOVEL RADIOTRACER TARGETING SYNAPTIC VESICLE PROTEIN 2A (SV2A)

**Warnock G[1]**, Aerts J[1], Bahri MA[1], Bretin F[1], Buchanan T[2], Klitgaard H[2], Mestdagh N[2], Valade A[2], Mercier J[2], Seret A[1], Luxen A[1], Salmon E[1], Plenevaux A[1]

[1] Cyclotron Research Centre, University of Liège, Belgium

[2] UCB Pharma, Brussels, Belgium

## INTRODUCTION & METHODS

**[18F]UCB-H is a fluorine-18 radiolabelled PET imaging agent with a nanomolar affinity for the human SV2A protein.** Synaptic vesicle protein 2A (SV2A) has been identified as the binding site of the antiepileptic levetiracetam (Keppra®) [1] and has thus been implicated in epilepsy. SV2 proteins are critical to proper nervous system function and have been demonstrated to be involved in vesicle trafficking. Their implication in epilepsy makes them an interesting therapeutic target, and additionally the widespread distribution of SV2A in particular may provide an opportunity to develop a PET-based measure of neuronal function in brain diseases.

- Male SD rats were provided by the Animal Facility of the GIGA-University of Liege (BE-LA 2610359). Mean (±SD) body weight at testing was 265±55 g.
- The animals were housed under standard 12h:12h light:dark conditions with food and water available ad libitum. All experimental procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Liege.
- The animals were imaged under isoflurane anaesthesia in a Siemens Concorde Focus 120 microPET scanner.
- Arterial input function was measured using an arteriovenous shunt method and beta microprobe system [2]. Blood samples taken from the shunt were used to quantify parent tracer and metabolite levels using HPLC and gamma counting.
- [18F]UCB-H was injected IV (3.8±0.54 mCi bolus, specific activity 8.5±0.86 Ci/µmol immediately after synthesis) and dynamic PET data acquired in list mode for 90 min. Images were reconstructed using filtered backprojection with correction for all physical effects except scatter.

## HIGHLIGHTS

**PET imaging revealed high uptake of [18F]UCB-H in brain and spinal cord, matching the expected homogeneous distribution of SV2A in the rodent brain [3].** Notably, the kinetics of [18F]UCB-H uptake in the brain were fast, peaking at up to 30 % ID/cm3 before a rapid decline.

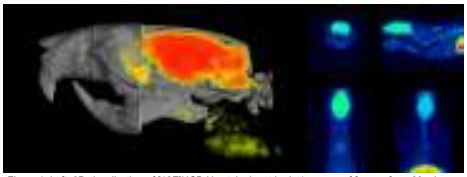

Figure 1: Left: 3D visualization of [18F]UCB-H uptake in rat brain (average of frames from 60 min PET scan) coregistered to CT scan; right: brain and spinal cord uptake of [18F]UCB-h in 2D planes (MIP lower right)

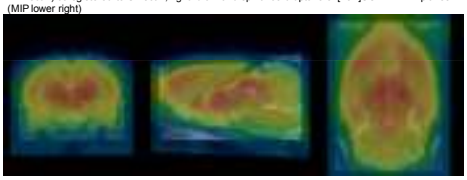

Figure 2: Averaged uptake of [18F]UCB-H in rat brain overlaid on standardized MRI template in PMOD software for automatic VOI definition and extraction of time-activity curves

## RESULTS & DISCUSSION

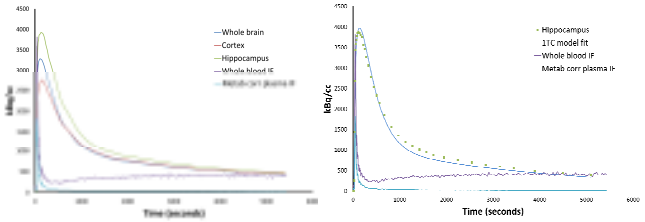


Figure 3: Left: Time-activity curves (TAC) for [18F]UCB-H uptake in whole brain, hippocampus and cortex representative regions, with matching beta microprobe-derived whole blood input function (IF) and corrected arterial plasma IF (corrected for plasma:whole blood [WB] ratio and plasma parent fraction – shown below); right: hippocampal TAC with corresponding 1-tissue compartment (1TC) model fit derived in PMOD (also shown – whole blood and corrected plasma IF).

The uptake of [18F]UCB-H in the brain over time was well fitted by a classical 1-Tissue Compartment model. Mean parameter estimates (mean±SD, n=7, whole brain VOI) were K1: 3.58±0.65 ml/cm3/min, k2: 0.21±0.03 min-1, Vt: 17.21±2.52 ml/cm3.

Metabolism of [18F]UCB-H in vivo followed a typical pattern of rapid initial metabolism followed by a reducing rate of metabolism over time, with less than 20% of the activity in plasma attributable to the parent compound after 30 minutes, and was highly reproducible between animals. One major metabolite (with low affinity for SV2a) was identified. The ratio of counts in whole blood samples to plasma samples was used to correct the beta microprobe-derived input function. A complex function over time was identified.

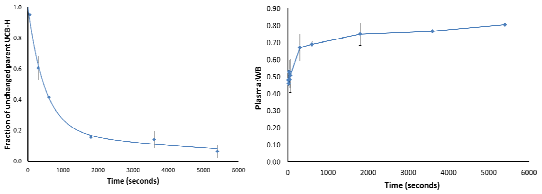


Figure 4: Left: parent fraction of unchanged [18F]UCB-H in plasma over the course of the scan time (data points show mean ± SD, n=2-3/time point); right: ratio of counts in plasma vs. whole blood (WB) in arterial blood samples over the course of the scan time (data points show mean ± SD, n=3-10/time point).

**Uptake of [18F]UCB-H was blocked by pretreatment with brivaracetam** (BRIV; 21 mg/kg IV, 10 min prior to [18F]UCB-H), a recently described high affinity SV2A ligand with a 20-fold higher affinity for SV2A than levetiracetam [3]. In contrast, pretreatment with ucb-100230-1, **a diastereoisomer of brivaracetam with 3200-fold lower affinity for SV2A [4], had no clear effect on the brain uptake of [18F]UCB-H.**

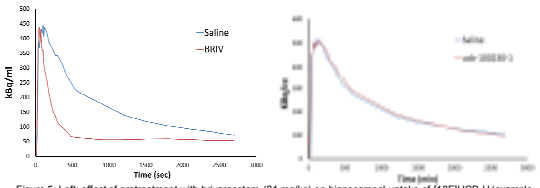


Figure 5: Left: effect of pretreatment with brivaracetam (21 mg/kg) on hippocampal uptake of [18F]UCB-H (example from a single subject – test-retest design, TAC scaled for injected dose); right: effect of ucb-100230-1 pretreatment on hippocampal uptake of [18F]UCB-H (example from a single subject – test-retest design, TAC scaled for injected dose).

## SUMMARY

**Our results indicate that [18F]UCB-H is a suitable radiotracer for the quantification of SV2A proteins in vivo** and for estimating target occupancy of drugs targeting SV2A. The measurement of SV2A occupancy by antiepileptic compounds will first be tested using levetiracetam (Keppra®). **This is the first PET tracer for in vivo quantification of SV2A**. The necessary steps for implementation of [18F]UCB-H production under GMP conditions have been completed and **first in human studies have already been performed** yielding interesting results.

REFERENCES

[1] Lynch, B.A. et al. (2004) PNAS 101(26):9861-6. [2[ Warnock, G. et al. (2011) EJNMMI Res. 1:13. [3] Janz, R. & Sudhof, T.C. (1999) Neuroscience 94(4):1279-1290. [4] Gillard, M. et al. (2011) Eur J Pharmacol 664:36-44.

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