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In vivo characterization of CNS penetrant KMO inhibitor using microdialysis coupled with a novel high content LC/MS/MS method

PsychoGenics

Redefining Drug Discovery Through Innovation

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Introduction

Degradation of the amino acid tryptophan (TRP) along the kynurenine pathway (KP) yields several neuroactive intermediates regulated by enzymes localized in astrocytes and microglial cells. Impairment of KP metabolism is functionally significant in a variety of diseases that affect the brain. It is hypothesized that CNS penetrant small molecules which inhibit kynurenine 3-monooxygenase (KMO) normalize central kynurenine dysregulation and will be beneficial for CNS disorders. We identified a CNS penetrant KMO inhibitor Compound A and characterized its effect on central and peripheral KP metabolites after systemic administration. We demonstrated that by extending the analyte capacity of an LC/MS/MS method, dedicated to KP metabolite, to include drug molecules we could simultaneously monitor KP metabolites and drug compounds in one injection. Utilizing this technique alongside *in vivo* microdialysis allows us to perform direct comparisons of drug levels and their impact on KP metabolites over a time course.

Methods

Animals, surgery and microdialysis

Male CD1 mice (25-30 g) were implanted with the guide cannula placed into the striatum. One week after surgery, a microdialysis probe was inserted for the microdialysis experiment. Microdialysis samples were collected for 6 hrs after administration of Compound A (30 and 100 mg/kg s.c.) and analyzed for Compound A and B, *L*-kynurenine (KYN), kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN), using LC/MS/MS. Terminal brain and plasma samples were taken for analysis of KP metabolites and compound exposure.

LC/MS/MS method for detection of metabolites

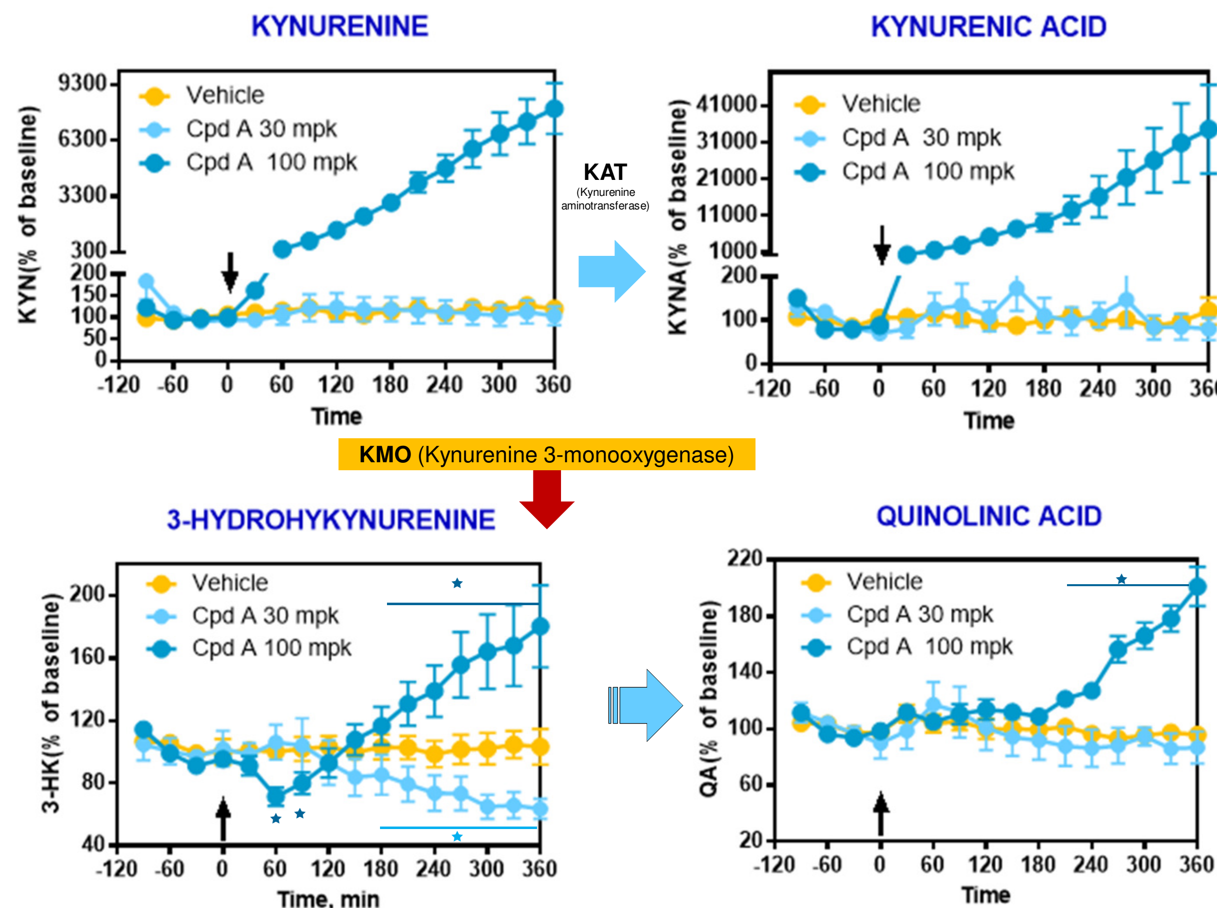
Standard curves were prepared using pure components including compound A and B, KYN, KYNA, 3-HK, 3-HAA, and QA dissolved in 0.2% acetic acid to correct for sample and instrument variability. Recovery and matrix impact on compound A and B were determined prior to analyses in plasma and brain samples to allow for exposure measures. Carbon 13 stable labeled KYN, 3-HK and QA and a deuterated analogue of KYNA were incorporated into plasma and brain samples as internal standards to correct for instrument and sample variability. Microdialysate measures were qualitative and so no internal standards or recovery/matrix tests were performed for these analyses.

Plasma or tissue blocks for homogenization were diluted 5 fold (w/v) by adding 0.2% acetic acid aqueous solution containing internal standards. Brain tissue samples were homogenized for 5 min. Diluted samples were filtered through 3 kDa filter. Resultant solutions or microdialysis samples were directly injected into a Waters (Milford, MA, USA). Acquity HPLC system equipped with an YMC™ ODS-AQ™ 2 mm × 100 mm, 3 μm particle column. Separated KP analytes were detected by a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters, Milford, MA, USA), operating in the MS/MS mode. Column and pre-column tubing were maintained at 40°C while eluting KP metabolites, compound A and B with a mobile phase consisting of an aqueous component (A: 0.5% formic acid in milliQ water) and an organic component (B: 1% formic acid in acetonitrile).

Quantification of KP metabolites in plasma and brain was performed by correction with the incorporated internal standards and by averaging of triplicate analysis. Limits of detection were determined based on a signal to noise ratio of >10 and found to be well below required limits for analysis of these analytes in rodent samples.

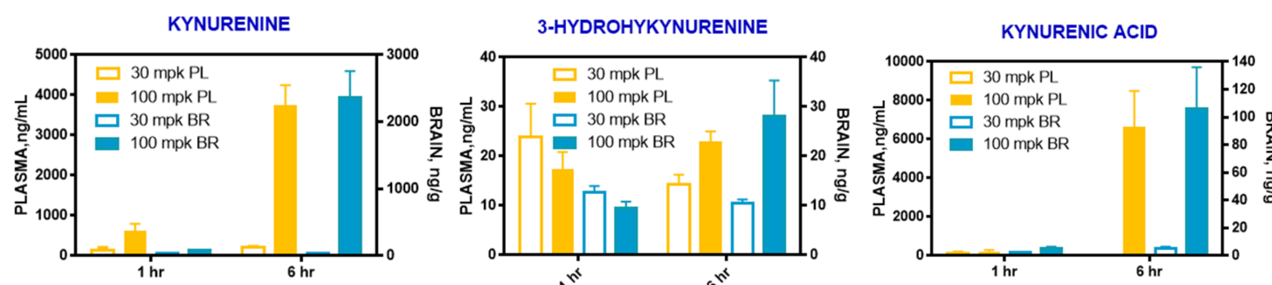
Results

Figure 1. Systemic administration of Cpd A affects KP metabolites in microdialysates collected from the striatum of mice



BrainLink (NL) polyacrylonitrile (PAN) probes (3 mm) were implanted into the striatum 16 hours before the study. Baseline samples were collected for two hours. Compound A was administered s.c. at time 0 (indicated by an arrow). Samples were collected every 30 min and analyzed for KP metabolites . For each animal, the average of the 3 consecutive samples collected immediately preceding compound administration was used as the basal level and was set to 100%. Data are presented as mean ± SEM. N=8 in each group. ★ - significantly different from the pre-dose baseline, P<0.05

Figure 2. Effect of systemic administration of Cpd A on brain and plasma KP metabolites



Animals participating in the microdialysis study and satellite animals were used for the 6 hr and 1 hr time point. Compound A was administered s.c. at 20 and 100 mg/kg. Animals were sacrificed, brain and plasma collected and processed for analyses of KP metabolites. N=3 for 1 hr group, n=8 for 6 hr group.

Figure 3. *In vivo* pharmacokinetics and PK/PD relationship of Cpd A studied by microdialysis

3-HK and Cpd A in microdialysates

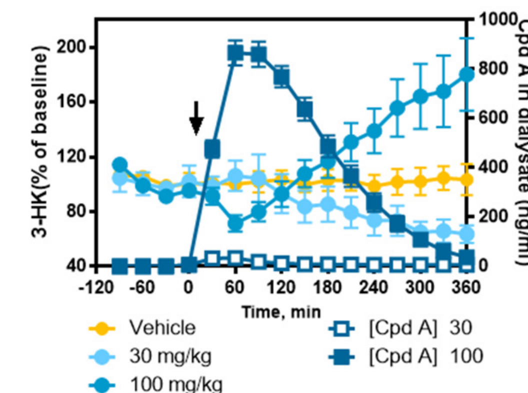
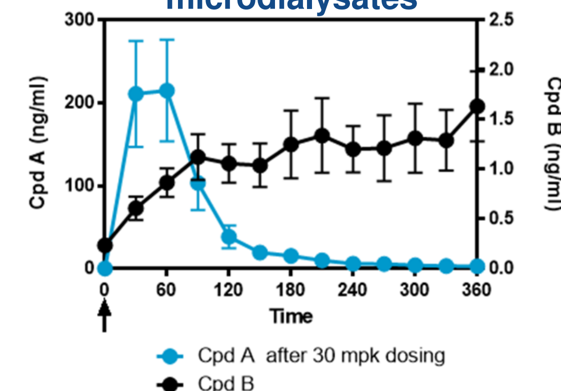


Figure 4. Formation of Cpd B, an active metabolite of Cpd A, is detected in microdialysates



Compound A was administered s.c. at time 0 (indicated by an arrow). Microdialysis samples were collected every 30 min and analyzed for 3-HK, Cpd A and Cpd B. Values were not adjusted for in vivo probe recovery..

Table 1. Brain and plasma exposure of Cpds A and B

Cpd A	Plasma ng/ml	Brain ng/g	B/P
30 mpk 1 hr	16437.9	1267.1	0.14
100 mpk 1 hr	135683.4	46364.3	0.35
30 mpk 6 hr	46.8	31.6	0.70
100 mpk 6 hr	1585.9	636.0	0.21

Cpd B	Plasma ng/ml	Brain ng/g	B/P
30 mpk 1 hr	143.3	2.4	0.1
100 mpk 1 hr	251.0	53.8	0.2
30 mpk 6 hr	20.3	4.4	0.2
100 mpk 6 hr	5675.8	339.8	0.1

Conclusions

- Cpd A, a CNS penetrant KMO inhibitor, affects both central and peripheral KP metabolism;
- Microdialysis coupled with LC/MS/MS method can detect changes related to the effect of the compound;
- Further studies of brain-penetrating modulators of KP may provide treatment options for CNS disorders