SSX, India

7th Annual Conference of the Society for the Study of Xenobiotics (SSX-2024)

MANIPAL ACADEMY of HIGHER EDUCATION BENGALURU CAMPUS (Institution of Eminence Deemed to be University)

Blood Brain Permeability and CNS Bioavailability of Targeted Protein Degraders

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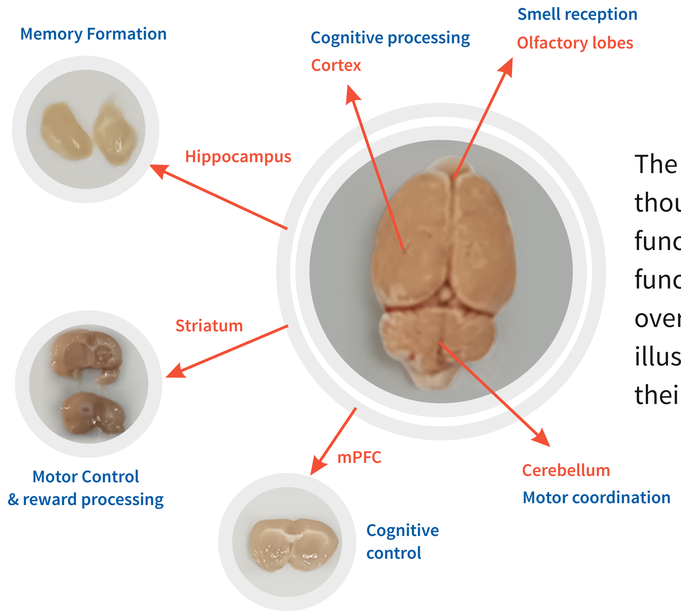
Poster ID PP-PKS-13

Introduction

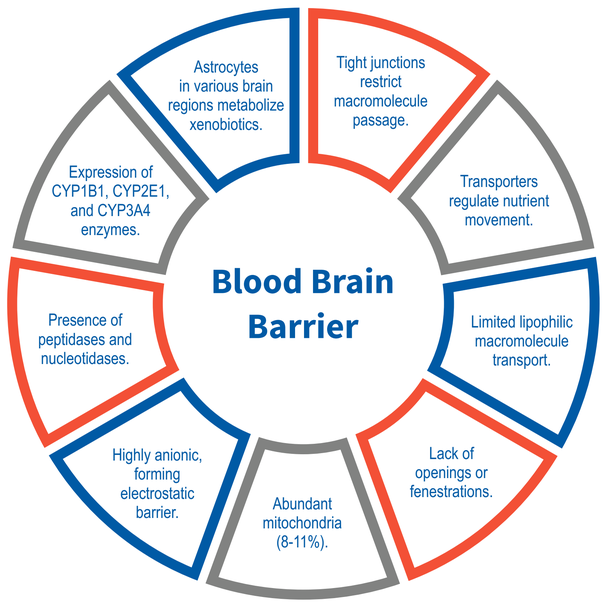
In the realm of central nervous system (CNS) diseases, the urgent need for novel treatments targeting proteinopathies has led to the emergence of Targeted Protein Degraders (TPDs). Despite concerns regarding their blood-brain barrier penetrability, some TPDs have shown promise in reaching the brain and cerebrospinal fluid, likely driven by their malleability to strike a balance between molecular size, aqueous solubility, oral bioavailability and blood brain barrier penetrability. This discussion tries to correlate the ADME properties of the TPDs with brain pharmacokinetic parameters and their likely interactions with drug transporters and thus unlocks some new dimensions to explore.

Brain : Part & Functions



The brain, a complex organ, regulates thoughts, memory, emotions, sensory function, motor skills, vision, lung function, temperature, satiety, and overall body homeostasis. This section illustrates various parts of the brain and their respective functions.

Blood Brain Barrier Components and Role



In light of the blood brain barrier structure and features, following critical attributes of CNS targeted drugs have been widely accepted

LogP = 1.5-2.7

Molecular weight cutoff = 400 daltons

Hydrogen bonding = 2 HBA & 1.5 HBD

Molecular volume and flexibility = Rotatable bond five or less

Polar surface area = 60–70 Å2, max limit 90 Å2

Charge = Net positive charge at pH 7–8

pKa = 4-10

Other = Tertiary nitrogen show a higher degree of brain permeation

Material and Methods

Plasma and brain exposure study of P-gp substrate loperamide was conducted in male CD-1 Mice with and without P-gp inhibitor elacridar. 48 mice were taken [6 at each time point i.e. 1hr, 4hr, 8hr and 24 hr x 2 groups]. Elacridar was formulated in 0.5% methyl cellulose and administered orally @100 mg/kg to group 2. The concentration was 10mg/ml and dose volume 10ml/kg. Vehicle [0.5% methyl cellulose] @10ml/kg was administered to group 1. 2hrs post elacridar dosing, loperamide formulated in DMSO (5%) + 20% HPβCD in Water (95%), was administered subcutaneously @1 mg/kg. At each time point, blood was collected from 6 animals of each group [1 and 2]. Post blood collection at respective timepoints, the mice were euthanized and cardiac perfusion performed through the insertion of 26g needle in the left ventricle. Animals were perfused with ~ 20mL ice cold PBS pH 7 @10mL/90 sec. After perfusion, the brain was removed from skull (without meninges) and stored at -80°C till bioanalysis.

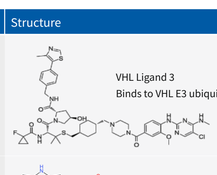
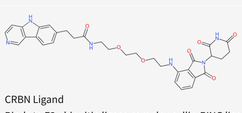
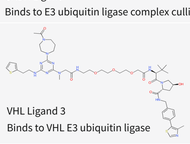
ADME Properties and PK Correlation

23 PROTACs were selected with molecular weight ranging from 725-825 daltons and Brain : Plasma ratio ranging from 0 to 11 to decipher the correlation between ADME properties and BBB permeability. All compounds were dosed at 1mg/kg IV to CD-1 mice. Brain collected at time point 8 hrs and Plasma: Brain ratio estimated.

Molecular Docking Analysis

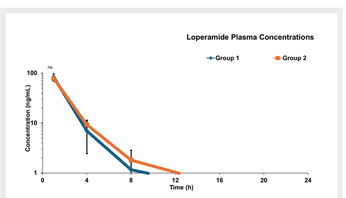
Following PROTACs, targeted for CNS indications, were taken for molecular docking analysis

Crystal structures of OATP1A2 and OATP2B1 are not available. The crystal structures of OATP1B1 and MCT-1 were retrieved from PDB and TPD structures were sourced from Pub Chem. To understand the nature of molecular interactions of TPDs, we carried out docking studies using swissdock, a web server that aim at extending the use of protein-small molecule docking software. We used manually curated protein structures . The transporter protein was assessed for the missing valences, hydrogens and was checked for any structural refinement, if needed. Based on the present coordinates, the TPDs were employed to molecular docking studies. UCSF Chimera was used for analyzing all the possible confirmations of the molecule. Discovery studio visualizer (v4.0) was used to visualize the interactions of receptor and ligand.

ID	Structure	Indication & Target	Properties
XL01126 (Ref: 1)	 VHL Ligand 3 Binds to VHL E3 ubiquitin ligase	Parkinson's disease. Leucine Rich Repeat Kinase 2 (LRRK2) degrader	tPSA: 194.3 Mol wt: 1019.7 XLogP: 5.05
QC-01-175 (Ref: 2)	 CRBN Ligand Binds to E3 ubiquitin ligase complex cullin-RING ligase 4	Alzheimer's disease. Degrader of pathogenic tau protein	tPSA: 171.82 Mol wt: 626.25 XLogP: 0.98
C004019 (Ref: 3)	 VHL Ligand 3 Binds to VHL E3 ubiquitin ligase	Alzheimer's disease selectively promotes tau protein degradation	tPSA: 302.39 Mol wt: 1034.48 XLogP: 1.81

Results

P-gp inhibition increases the content of P-gp substrate in Brain



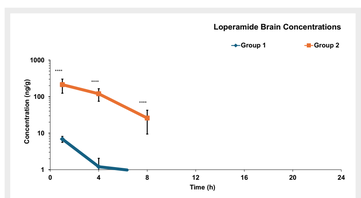
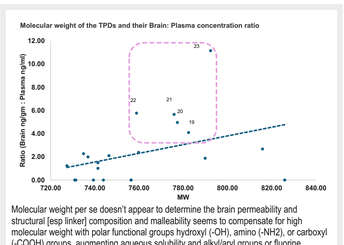


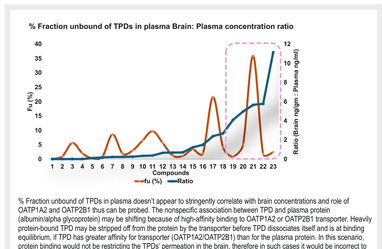
Table: Loperamide - Tissue: Plasma concentration

Time Points (h)	Group 1 (Control)			Group 2 (P-gp Inhibitor)		
	Mean	SD	N	Mean	SD	N
1	0.08	0.02	6	2.71	1.15	6
4	0.15	0.11		12.93	4.81	
8	1.97	1.29		24.46	28.26	
24	NC	NA		NC	NA	

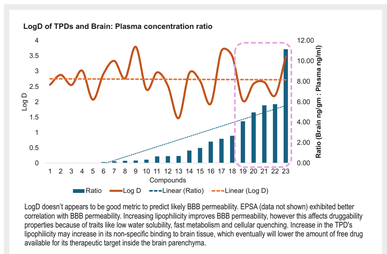
Unpaired t-test, F- test for comparison



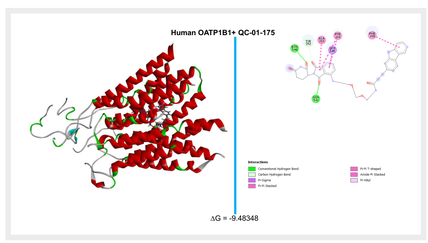
Molecular weight per se doesn't appear to determine the brain permeability and structural [esp linker] composition and malleability seems to compensate for high molecular weight with polar functional groups hydroxyl (-OH), amino (-NH2), or carboxyl (-COOH) groups, augmenting aqueous solubility and alkyl/aryl groups or fluorine substitution adjacent to -OH group conferring lipophilic traits.

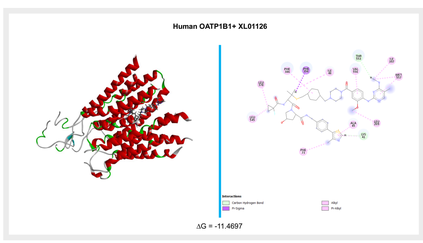


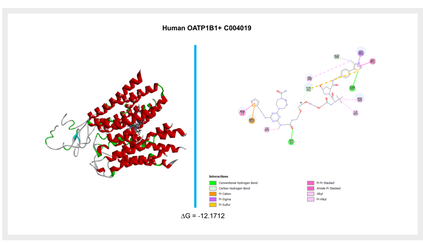
% Fraction unbound of TPDs in plasma doesn't appear to stringently correlate with brain concentrations and role of OATP1A2 and OATP2B1 thus can be probed. The nonspecific association between TPD and plasma protein (albumin/alpha glycoprotein) may be shifting because of high-affinity binding to OATP1A2 or OATP2B1 transporter. Heavily protein-bound TPD may be stripped off from the protein by the transporter before TPD dissociates itself and is at binding equilibrium, if TPD has greater affinity for transporter (OATP1A2/OATP2B1) than for the plasma protein. In this scenario, protein binding would not be restricting the TPDs' permeation in the brain, therefore in such cases it would be incorrect to use fu values obtained in-vitro at equilibrium to predict the drug available for brain penetration (**transporter-induced protein binding shift**). Also, CYP1B1 and CYP2U1 liability can be probed.

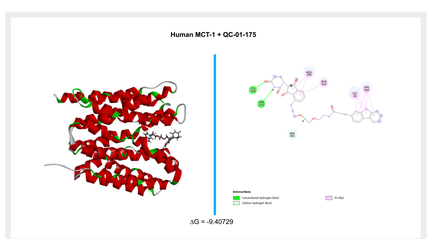


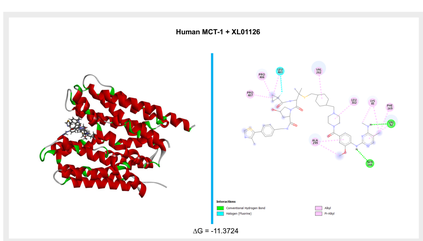
LogD doesn't appears to be good metric to predict likely BBB permeability, EPSA (data not shown) exhibited better correlation with BBB permeability. Increasing lipophilicity improves BBB permeability, however this affects druggability properties because of traits like low water solubility, fast metabolism and cellular quenching. Increase in the TPD's lipophilicity may increase in its non-specific binding to brain tissue, which eventually will lower the amount of free drug available for its therapeutic target inside the brain parenchyma.

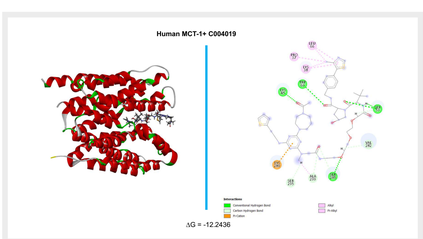












Discussion

It's an enduring issue to deliver macromolecular therapeutics into CNS due to the limitations posed by BBB. Chemical macromolecules the "TPDs" are large molecules "beyond Rule of 5" (bRo5) chemical space. To ameliorate the CNS disease condition, the first hurdle for TPDs is their structural composition and size which limits the BBB permeability. The second obstacle is the brain cells which are relatively difficult to transfect. The third stumbling block for TPDs is harnessing the downregulated proteasome machinery (as observed in various CNS diseases) to eliminate the aberrant proteins and exhibit reasonable clinical therapeutic efficacy.

Herein we tried to ascertain the correlation of ADME properties of TPDs with their Brain concentrations at 8hrs. The ADME properties widely perceived as indicator of BBB permeability doesn't seem to correlate well in case of TPDs. Further, we attempted to explore interactions of Three PROTACs (targeted for CNS indications) with OATP1B1 and MCT-1 Transporters. The TPDs exhibited reasonable interaction with both the transporters with binding energy ranging between -9.4 to -12.24.

1. Discovery of XL01126: A Potent, Fast, Cooperative, Selective, Oral Bioavailable and Blood Brain Barrier Penetrant PROTAC Degrader of Leucine Rich Repeat Kinase 2. Liu, X.; Kalogeropulou, A.F.; Domingos, S.; Makukhin, N.; Nirujogi, R.S.; Singh, F.; Shpiro, N.; Saalfrank, A.; Sammler, E.; Ganley, I.G.; et al. J. Am. Chem. Soc. 2022, 144, 16930–16952.
2. Targeted degradation of aberrant tau in frontotemporal dementia patient-derived neuronal cell models. Silva MC, Ferguson FM, Cai Q, Donovan KA, Nandi G, Patnaik D, Zhang T, Huang HT, Lucente DE, Dickerson BC et al. (2019). Elife, 8: e45457.
3. A novel small-molecule PROTAC selectively promotes tau clearance to improve cognitive functions in Alzheimer-like models. Wang W, Zhou Q, Jiang T, Li S, Ye J, Zheng J, Wang X, Liu Y, Deng M, Ke D et al.. (2021) Theranostics, 11 (11): 5279-5295.
4. Healthy aging and the blood–brain barrier. William A. Banks , May J. Reed, Aric F. Logsdon, Elizabeth M. Rhea and Michelle A. Erickson. Nature Aging. VOL 1. March 2021. 243–254.

Our Future Directions

It would be interesting to decipher the mechanism(s)/route(s) TPDs utilizing to cross BBB. Accordingly, following avenues are being explored at Aragen:

1. The likelihood of passive diffusion of TPDs through post-capillary venules (PCVs) is being evaluated. PCVs are the point-of-least resistance at the BBB, and compared to capillaries, PCVs are the key locus for transcytosis-mediated brain delivery of therapeutic nanoparticles. Further, there is ample evidence to support the notion that PCVs are the preferred site of extravasation for leukocytes, tumor cells, and parasites. Capillaries' slowest blood velocity could be lengthening the time TPDs spend interacting with venular endothelial cells which contain intracellular machinery that can aid TPDs get internalized.
2. The passive diffusion propensity is being assessed by comparing brain concentrations of TPD in young (2 months) V/s aged (16 months) rat. Both, the number of tight junctions and total length per capillary decrease in aged rats. (Ref- 4)
3. The ability of OATP1A2, OATP2B1, MCT-1, OATP1B1 to transport TPDs across the plasma membrane is being evaluated using transporter overexpressing cell lines. This is expected to have implications from cancer cell targeting perspective too as OATP1A2 and MCT-1, for example, is highly expressed in tumor cells (breast, prostate, bone cancer to name a few).
4. CYP1B1 in CNS breaks down a number of endogenous substances including retinol, prostanoids, estradiol and melatonin. CYP1B1 liability of investigational TPDs is being assessed to determine metabolic stability in CNS and obtain holistic view of Brain pharmacokinetics.
5. Novel Mechanism: is being explored in unilateral ICA ligation rat model, infusing TPDs at 1ul/min and sampling through contralateral jugular vein (10ul/10min) followed by estimation of conc in specific regions of brain and assessing the differential gene expression through NGS.