P-glycoprotein (Abcb1a/Mdr1a) limits brain exposure to anti-cancer drug candidate R-roscovitine/seliciclib.

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INTRODUCTION: Seliciclib (R-Roscovitine) a cyclin – dependent kinase inhibitor, is a promising drug candidate to treat a variety of cancer. The aim of our study was to investigate the effect of a specific ABCB1 inhibitor (PSC-833) on the brain distribution of seliciclib in vivo in anesthetized and freely moving mice. For making in vivo-in vitro (IVIV) correlation a mouse Mdr1a expressing LLC-PK1 monolayer cell line was applied.

METHODS: To investigate the brain exposure of seliciclib (50 mg/kg i.p.) dual-probe microdialysis technique was applied in anesthetized, and singe-probe microdialysis in freely moving mice. PSC-833 (10 mg/kg i.p.) was applied as a pretreatment. Concentration-time profiles were determined and AUCbrain/AUCblood was calculated. For IVIV correlation seliciblib and LY335979 + seliciclib were tested in LLC-PK1-mock and LLC-PK1-mMdr1a.

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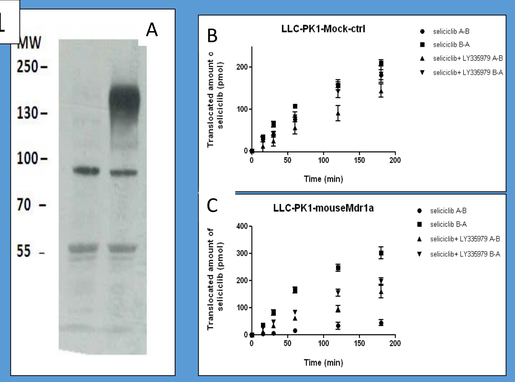

Fig.1 In vitro characterization of LLC-PK1-mouseMdr1a cell line in Western blotting (A), and demonstration of vectorial transport of seliciclib (B and C) in monolayer efflux assays in LLC-PK1-mMdr1a and LLC-PK1-Mock-ctrl cells

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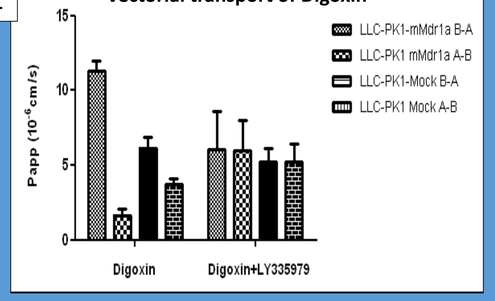

Fig.2 Effect of Mdr1a on the apparent permeability of digoxin determined by bidirectional transport across LLC-PK1-mMdr1a and control monolayers in the presence of specific inhibitor LY335979.

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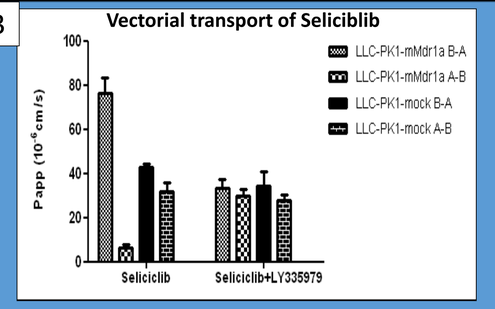

Fig.3 Effect of Mdr1a on the apparent permeability of seliciclib determined by bidirectional transport across LLC-PK1-mMdr1a and control monolayers in the presence of specific inhibitor LY335979.

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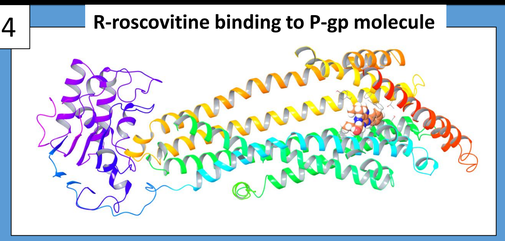

Fig.4. Docking of R-roscovitine to 3D structure of P-pg molecule

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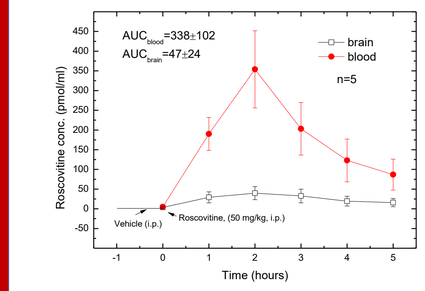

Fig 5. Concentration-time profiles of R-roscovitine in dialysate samples from brain and blood of anesthetized mice

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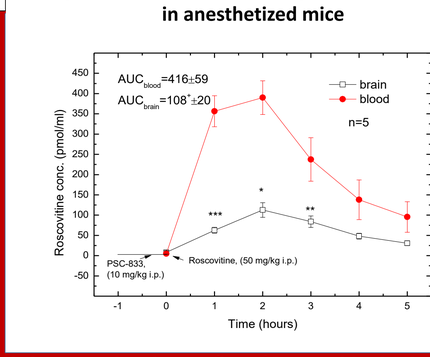

Fig 6. Concentration-time profiles of R-roscovitine in dialysate samples from brain and blood of anesthetized mice in presence of PSC-833

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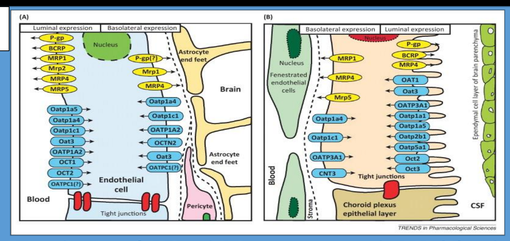

Fig 7. Localization of ABC and SLC transporters at the BBB (A) and BCSFB (B) (From: Chan GN et al, TIPS, 2013, 34:361-72.)

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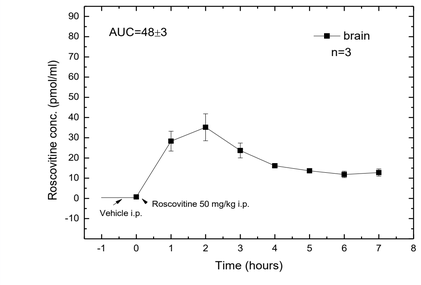

Fig 8. Concentration-time profiles of R-roscovitine in dialysate samples from brain of awake mice

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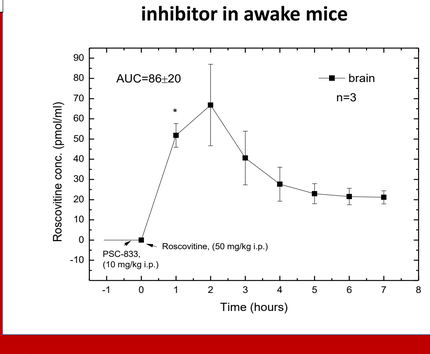

Fig 9. Concentration-time profiles of R-roscovitine in dialysate samples from brain of awake mice in presence of PSC-833

CONCLUSIONS:

- **Abcb1a significantly decreases seliciclib premeability in vitro in LLC-PK1 cells.**
- **Abcb1a is partly responsible for limited brain exposure of seliciclib/R-roscovitine in vivo in mice**

ACKNOWLEDGEMENTS:
The 3D docking of R-roscovitine to P-gp molecule was performed by Csaba Magyar, PhD, at the Institute of Enzymology, MTA TTK, Budapest