A Useful Relationship between *In Vivo Rat* Kp Brain and *In Vitro* Caco-2 Cells Efflux Ratio

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ABSTRACT

Introduction: CNS targeting is a challenging area, where both in vitro and in vivo approaches are needed at the different R&D stages. When CNS exposure is a key parameter to optimize, *in vitro* surrogates are actively looked for. This search is not always successful for new chemical series until the limiting factor(s) of brain penetration are elucidated (transport, passive permeability, protein binding, etc.). In one of the Inventiva projects, we established an interesting *vitro/vivo* correlation between Caco-2 cells and rat Kp Brain. **Method: *In vitro* Caco-2**. Compounds were tested at 10 µM in a 96-well permeable plate seeded with Caco-2 cells. The medium was the same in apical and basolateral sides: Hanks' Balanced Salt Solution (HBSS) + 5 mM Hepes + bovine serum albumin 1%, pH 7.4. The assay was performed with a robotic platform (Caliper-Perkin Elmer system). After incubation for 2 hours, the concentrations of tested compound was measured in both sides by LC-MS/MS (API4000 Qtrap, AB Sciex). Permeability in both directions (apical to basolateral and basolateral to apical) was assessed to determine the efflux ratio. ***In vivo* rat Kp Brain**. Four male Wistar rats were used per test compound. Under anesthesia, a catheter was chronically implanted in the jugular vein, tunneled subcutaneously, exteritorized at the back of the neck, tethered with a saddle maintained with a harness and attached to a swivel device at the top of the cage (Phymep SARL). The system was connected to a syringe pump (Harvard apparatus). Test compound was solubilized at 0.33 mg/mL in saline containing 2% Cremophor EL and was infused to the animals during 4 h. Then, the animals were humanely sacrificed to sample blood and brains. Test compound concentrations were determined by LC-MS/MS analysis, and Kp brain was calculated as brain concentration/plasma concentration. **Results:** When the Kp Brain was plotted as a function of the *in vitro* Efflux Ratio (ER), a threshold appeared. For ER values > c.a. 0.6 (i.e. influx < 1.7), Kp Brain values were < 0.3 or even << 0.3. For ER values ≤ c.a. 0.6 (i.e. influx ≥ 1.7), 70% of Kp Brain values were > 0.3 and 30% were between 0.1 and 0.3. The Caco-2 ER was then used as a surrogate, and the relationship was confirmed all along when *in vivo* Kp Brain were determined. **Conclusion:** For certain chemical series, the Caco-2 Efflux Ratio is a good Kp Brain surrogate that can help chemical design and screening. The Caco-2 test described here used a pH 7.4 buffer with 1% BSA both in the apical and basolateral sides. The underlying mechanisms of these compound penetrations are under investigation both for the Brain and for the Caco-2 cells.

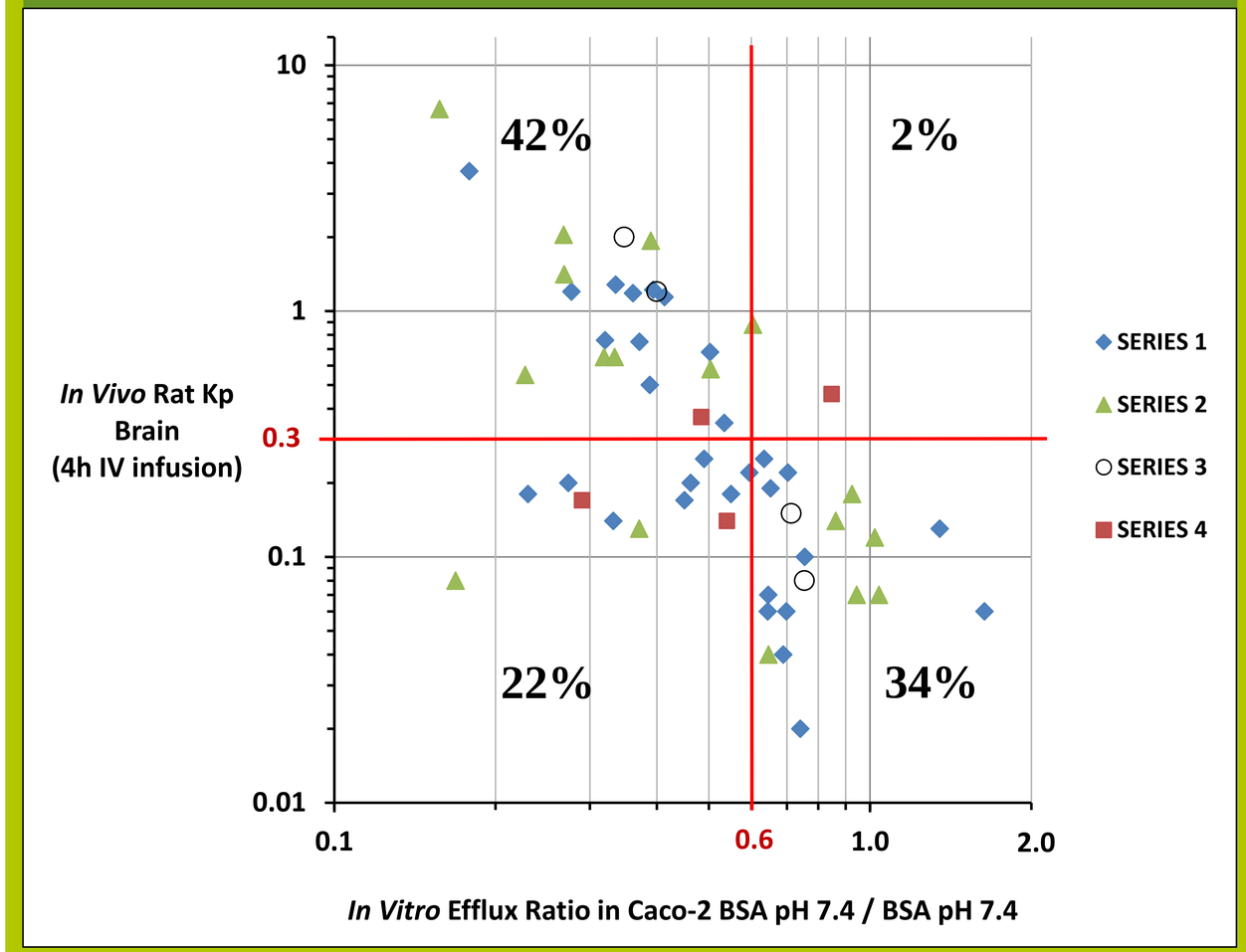
MATERIAL AND METHODS

***In vitro* Caco-2**: Compounds were tested at 10 µM in a 96-well permeable plate seeded with Caco-2 cells. The medium was the same in apical and basolateral sides: Hanks' Balanced Salt Solution (HBSS) + 5 mM Hepes + bovine serum albumin 1%, pH 7.4. The assay was performed with a robotic platform (Caliper-Perkin Elmer system). After incubation for 2 hours, the concentrations of tested compound was measured in both sides by LC-MS/MS (API4000 Qtrap, AB Sciex). Permeability in both directions (apical to basolateral and basolateral to apical) was assessed to determine the efflux ratio (BA Papp / AB Papp).

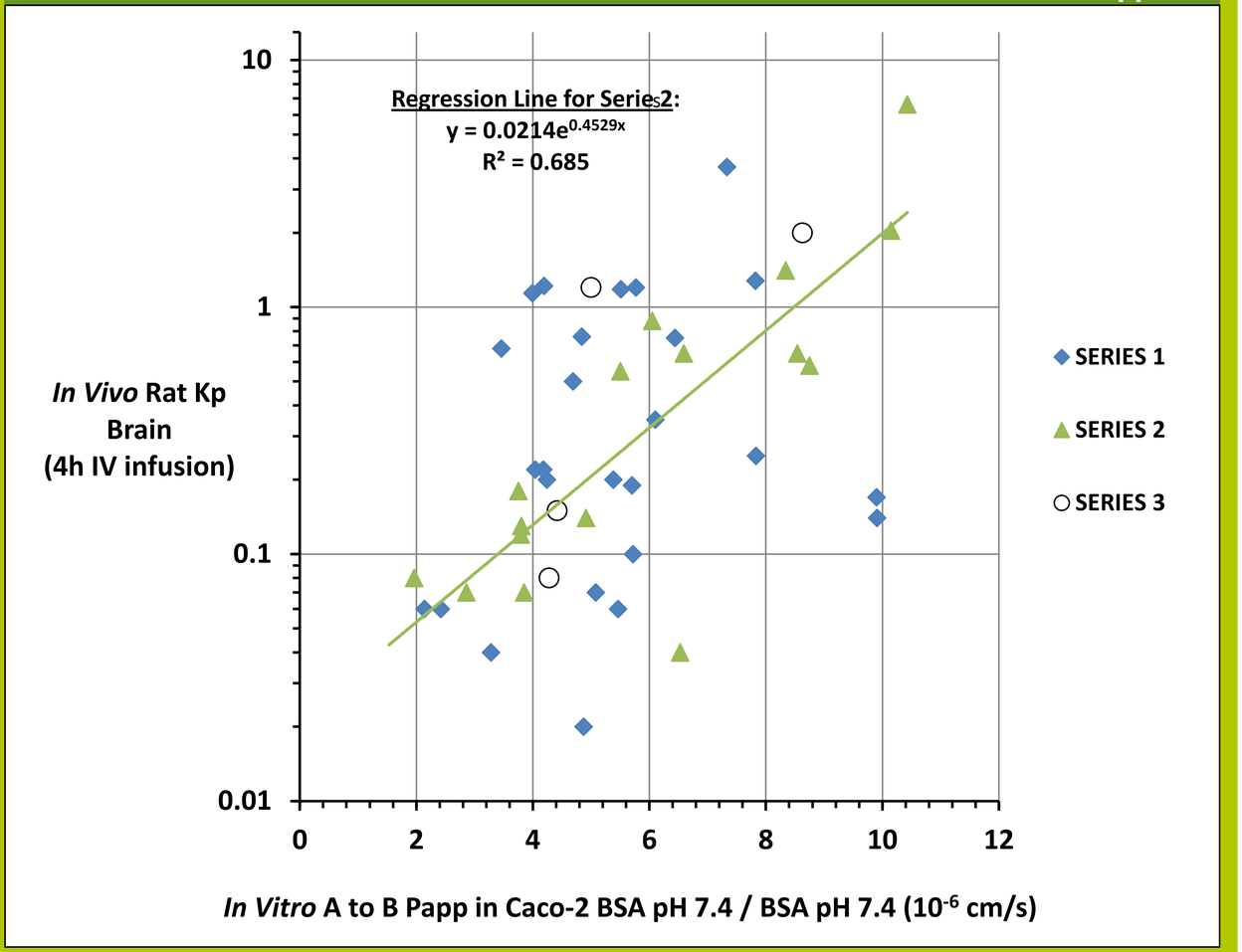
***In vivo* rat Kp Brain**: Four male Wistar Rats were used per test compound. Under anesthesia, a catheter was chronically implanted in the jugular vein, tunneled subcutaneously, exteritorized at the back of the neck, tethered with a saddle maintained with a harness and attached to a swivel device at the top of the cage (Phymep SARL). The system was connected to a syringe pump (Harvard apparatus). Test compound was solubilized at 0.33 mg/mL in saline containing 2% Cremophor EL and was infused to the animals during 4 h. Then, the animals were humanely sacrificed to sample blood and brains. Test compound concentrations were determined by LC-MS/MS analysis, and Kp brain was calculated as brain concentration/plasma concentration.

RESULTS

RELATIONSHIP BETWEEN *IN VIVO* KP BRAIN AND *IN VITRO* CACO-2 EFFLUX RATIO



RELATIONSHIP BETWEEN *IN VIVO* KP BRAIN AND *IN VITRO* CACO-2 A to B Papp



DISCUSSION AND CONCLUSION

The *in vitro* Efflux Ratio (ER) was identified as an efficient predictor regarding *in vivo* brain penetration, with more than 75% (42% + 34%) success rate. This early measure was advantageously used to prioritize the progression of our compounds further down the screening cascade:

- An *in vitro* ER value below or equals to 0.6 was associated with a high probability for a good *in vivo* Kp Brain (value > 0.3 and >> 0.3), for all our series except series 4.
- Further investigations are undergoing to refine the model and understand why 22% of the molecules are classed as false positive (ER < 0.6 with Kp values between 0.1 and 0.3 only).
- Almost all the compounds exhibiting an *in vitro* ER value > 0.6 (only 1 outlier out of 20 molecules) had *in vivo* Kp values below 0.3 or even far below 0.3.
- A log-linear relationship between Caco-2 A to B Papp and Kp Brain was observed only for Series 2. The Papp was therefore less informative than the ER to predict compounds regarding the brain penetration.

In conclusion for the tested chemical series, the Caco-2 Efflux Ratio (BSA pH 7.4 / BSA pH 7.4) was a good Kp Brain surrogate to select and minimize the number of molecules to be tested *in vivo*.