One Hour Pre-Infusion followed by Four Hours Co-Infusion of Elacridar as a Tool to Identify the Involvement of P-gp and BCRP in the Brain Penetration of Test Compounds in Rats

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ABSTRACT

Introduction: Active efflux can be an important hurdle for CNS targeting, and dedicated *in vivo* studies must be performed at some stage of the research and development. Elacridar is a strong and, as far as we currently know, relatively specific inhibitor of P-gp and BCRP, two main efflux transporters. However, published protocols for its use *in vivo* differ markedly. We developed and validated a method that approaches steady-state and that can be used routinely within one day of administration. **Method:** Eight male Wistar rats were used per test compound: four for the test compound alone and four for the test compound with Elacridar. 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, Paris, France). The system was connected to a syringe pump (Harvard apparatus, Les Ulis, France). We developed a vehicle that was well tolerated by the animals, that was able to solubilize Elacridar at 1 mg/mL + test compound at 0.33 mg/mL during at least 5 h, and that was easy to prepare routinely. The administration started with 1 h pre-infusion of the Elacridar solution, followed by 4 h co-infusion of Elacridar + test compound. Then, the animals were humanely sacrificed to sample blood and brains. Elacridar and test compound concentrations were determined by LC-MS/MS analysis, and Kp brain was calculated as brain concentration/plasma concentration. **Results:** As expected, the Kp brain of Digoxin was strongly increased in the presence of Elacridar, therefore validating the method. The Kp brain of the IVA test compound was 0.16 ± 0.03 w/o Elacridar and 0.24 ± 0.05 with Elacridar. These two values are not different from the mean historical Kp of this molecule: 0.22 ± 0.08 (95% confidence interval, n=6 experiments). **Conclusion:** The method is well suited to determine the involvement of P-gp and BCRP in the brain penetration of test compounds *in vivo*. Data for Elacridar alone will also be shown and discussed.

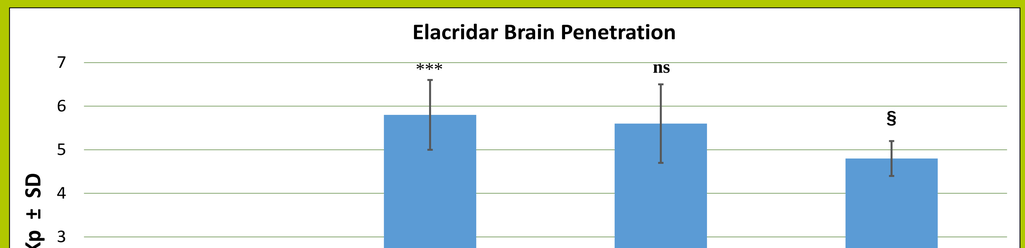
MATERIAL AND METHODS

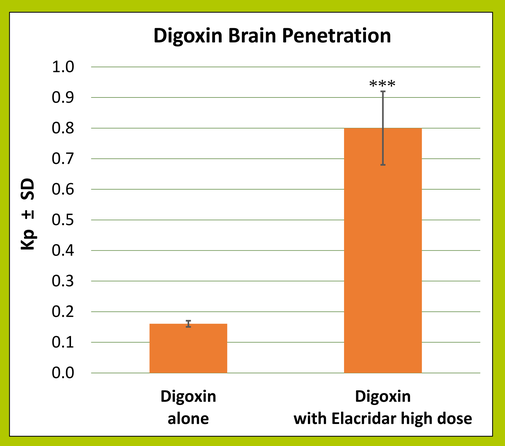
Animals: Male Wistar rats 250-350 g, Janvier France ; 4 per compound alone and 4 for compound + Elacridar. **Surgery and Analgesia:** Rats were anaesthetized by ketamine/xylazine *i.m.* One drop of Ocry-gel (Laboratoire TVM, France) was dropped on each eye at the beginning and at the end of the surgery to avoid eye-drying. A survival blanket was used. A sterile catheter was implemented in one 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 (all from Phymep SARL, Paris, France). The system was connected to a syringe pump (Harvard apparatus, Les Ulis, France). Heparinized saline was used at different steps. To prevent post operative pain, buprenorphine 25 µg/kg was administered before the operation. Buprenorphine was expected to be cleared after 24 h. **Formulation:** We developed a proprietary vehicle that was well tolerated by the animals, that was able to solubilize Elacridar at 1 mg/mL + test compounds up to 0.33 mg/mL during at least 5 h, and that was easy to prepare routinely. **Infusion:** The administration started with 1 h pre-infusion of the Elacridar solution, followed by 4 h co-infusion of Elacridar + test compound. The infusion flow was 50 µL/min/kg. **Analysis:** At the end of infusion the animals were anaesthetized by isoflurane/O2 and blood and brain were sampled. Elacridar and test compound concentrations in plasma and brain were determined by LC-MS/MS analysis (see Results for the limits of quantification), and Kp brain was calculated as brain concentration/plasma concentration. Plasma clearance was calculated as infusion rate / end plasma concentration. Statistical tests were performed with JMP software (SAS, France), testing first for variances equality.

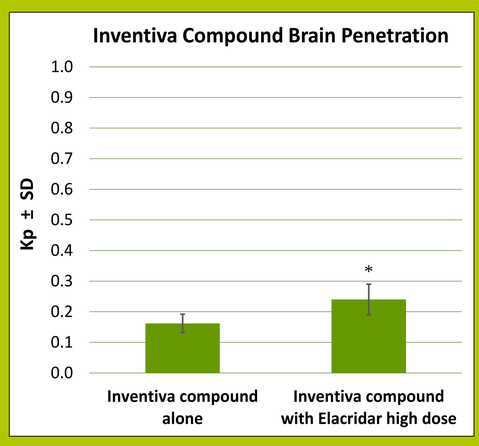
RESULTS

Compound	Target Dose (mg/kg)	Meas. Dose (mg/kg)	Perf. Time (h)	Perf. Rate (mg/h/kg)	Plasma Cend (ng/mL)	Plasma CL[1] (mL/min/kg)	Brain Cend (ng/g)	Kp Brain	SD CV N
Elacridar low dose	1.5	1.5	5	0.3	229	22	134	**0.60**	0.12 21% 4
Elacridar high dose	15	13	5	2.6	2 402	18	13 733	**5.8*** **	0.8 14% 4
Digoxine	0.24	0.24	4	0.06	46	18	7.3	**0.16**	0.01 6% 3
Digoxine + **Elacridar**	0.24 15	- -	4 5	0.06 3.0	57 2 243	14 21	45 12 532	**0.80*** ** **5.6[ns]**	0.12 15% 4 0.9 16% 4
Inventiva cpd	4.0	3.6	4	0.9	12 556	1.0	1 992	**0.16**	0.03 21% 4
Inventiva cpd + **Elacridar**	4.0 15	3.9 -	4 5	1.0 3.0	11 783 2 781	1.1 17	2 751 13 222	**0.24*** **4.8[§]**	0.05 20% 4 0.4 8% 4

[1]: assuming steady-state is reached after the infusion. **ns**: not significant ($p > 0.05$). ***: $p < 0.002$, Welsh test for inequal variance. *: $p = 0.04$, One factor ANOVA for equal variances. §: $p = 0.03$, One factor ANOVA for equal variances.
Limit of LC-MS/MS quantification in plasma and in brain: Elacridar 6.0 ng/mL and 1.8 ng/g; Digoxine 6.0 ng/mL and 3.5 ng/g; Inventiva cpd 20 ng/mL and 30 ng/g







DISCUSSION AND CONCLUSION

The vehicle was well tolerated, easy to prepare and with a high solubilizing capacity. The objective of the 1 h pre-infusion with Elacridar was to start the inhibition P-gp and BCRP before the administration of the test compound in order to maximize the effect and reduce the infusion time. The Kp brain variability was low, with CV's between 8% and 21%. Plasma clearances of Inventiva compound, Elacridar and Digoxine were not modified when administered alone or in combination.

Elacridar Kp value, when given on its own, was of 0.60 at the low dose and increased almost by ten-fold to 5.8 at the high dose. This indicated that Elacridar inhibited / saturated its own efflux at the blood brain barrier, as previously shown (1).

Digoxine Kp value was 0.16 and increased significantly by 5-fold to 0.80 in the presence of Elacridar, in agreement with published data (2). This showed that Elacridar inhibited the P-gp related efflux of Digoxine at the blood brain barrier thereby validating the method. Digoxine did not change the Elacridar Kp brain.

Inventiva compound Kp value was 0.16. It increased weakly but in a statistically significant manner by 1.5-fold to 0.24 in the presence of Elacridar hence demonstrating that the Inventiva compound was not a P-gp or BCRP substrate, or only marginally. *In vitro* experiments confirmed that it was not a substrate of P-gp or BCRP efflux pumps. Elacridar Kp value decreased slightly by 17% in the presence of Inventiva compound. Such a slight effect was most probably of no biological significance.

In conclusion, we demonstrated with these rats *in vivo* studies that this test is well suited to identify, in steady-state conditions, the P-gp and BCRP involvement in the brain penetration of test compounds.

REFERENCES

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