Transfer of Rhodamine-123 into the Brain and Cerebrospinal Fluid of Fetal, Neonatal and Adult Rats

Liam Koehn (lkoehn@wihri.org)
University of Melbourne https://orcid.org/0000-0003-3845-2953
Katarzyna M Dziegielewska
University of Melbourne - Parkville Campus: University of Melbourne
Mark D Habgood
The University of Melbourne - Parkville Campus: University of Melbourne
Yifan Huang
University of Melbourne - Parkville Campus: University of Melbourne
Norman R Saunders
University of Melbourne - Parkville Campus: University of Melbourne

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Research

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Abstract

Background: Adenosine triphosphate binding cassette transporters such as P-glycoprotein (PGP) play an important role in drug pharmacokinetics by actively effluxing their substrates at barrier interfaces, including the blood-brain, blood-cerebrospinal fluid (CSF) and placental barriers. For a molecule to access the brain during fetal stages it must bypass efflux transporters at both the placental barrier and brain barriers themselves. Following birth, placental protection is no longer present and brain barriers remain the major line of defense. Understanding developmental differences that exist in the transfer of PGP substrates into the brain is important for ensuring that medication regimes are safe and appropriate for all patients.

Methods: In the present study PGP substrate rhodamine-123 (R123) was injected intraperitoneally into E19 dams, postnatal (P4, P14) and adult rats. Naturally fluorescent properties of R123 were utilized to measure its concentration in blood-plasma, CSF and brain by spectrofluorimetry (Clariostar). Statistical differences in R123 transfer (ratios between tissue and plasma concentrations) were determined using Kruskal-Wallis tests with Dunn's corrections.

Results: Following maternal injection the transfer of R123 across the E19 placenta from maternal blood to fetal blood was around 20%. Of the R123 that reached fetal circulation 41% transferred into brain and 38% into CSF. The transfer of R123 from blood to brain and CSF was lower in postnatal pups and decreased with age (brain: 43% at P4, 22% at P14 and 9% in adults; CSF: 8% at P4, 8% at P14 and 1% in adults). Transfer from maternal blood across placental and brain barriers into fetal brain was approximately 8%, similar to the transfer across adult blood-brain barriers (9%). Following birth when placental protection was no longer present, transfer of R123 from blood into the newborn brain was significantly higher than into adult brain (3 fold, $p<0.05$).

Conclusions: Administration of a PGP substrate to infant rats resulted in a higher transfer into the brain than equivalent doses at later stages of life or equivalent maternal doses during gestation. Toxicological testing of PGP substrate drugs should consider the possibility of these patient specific differences in safety analysis. Trial Registration: N/A.

Full Text

This preprint is available for download as a PDF.

Figures

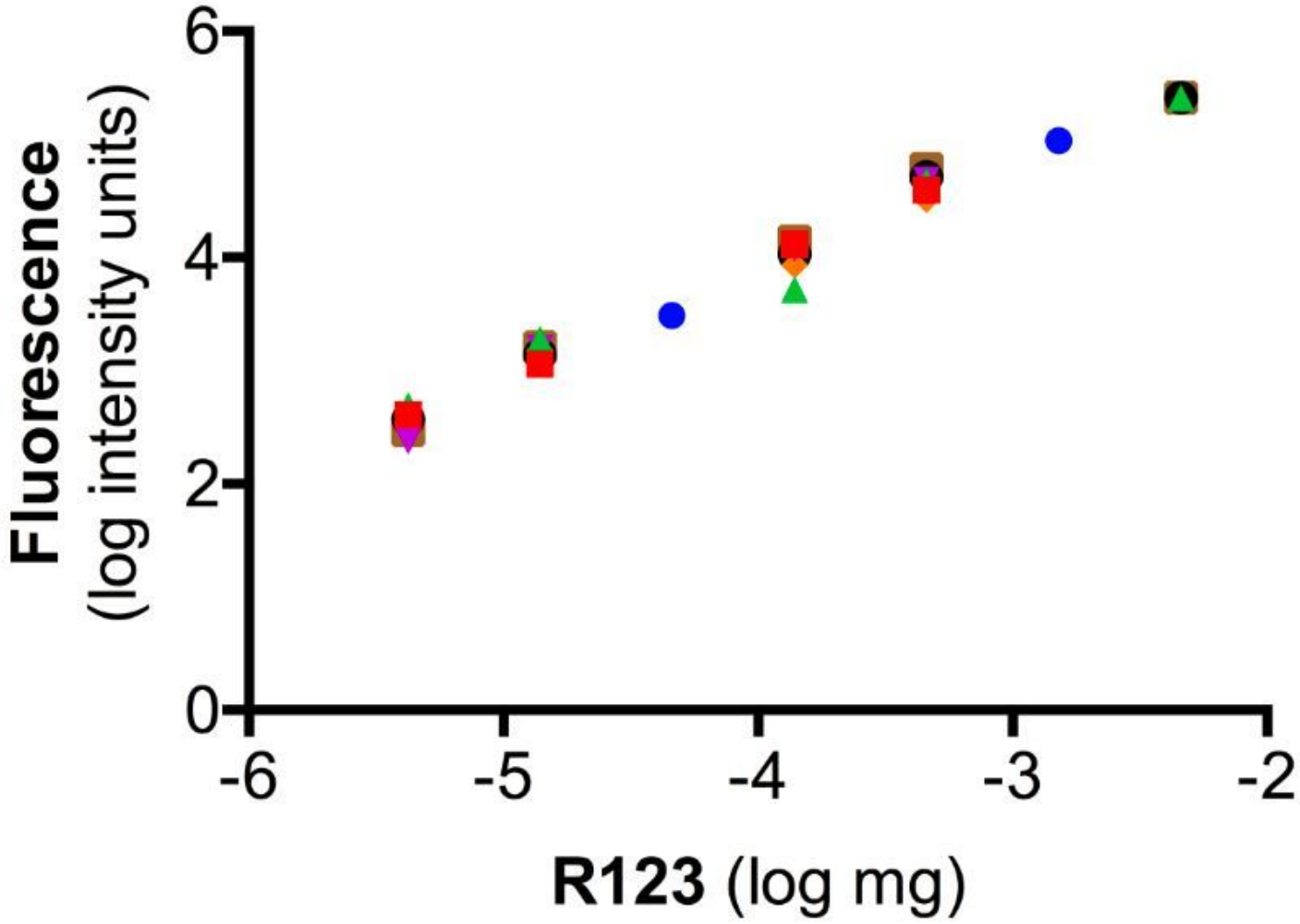

Figure 1

Linear range of R123 detection. Clariostar fluorescence readings of rhodamine-123 (R123) measured as sample extracts in 30µl Corning microplate (see Methods). Samples analyzed were P4 plasma (blue circle), P14 plasma (red square), adult plasma (green triangle), P4 brain extract (purple inverted triangle), P14 brain extract (orange diamond), adult brain extract (black large circle) and adult CSF (brown square). Note the similarity (overlap) of samples over the linear range. Representative equation for all data points: y=0.96x+7.8; R2 =0.98.

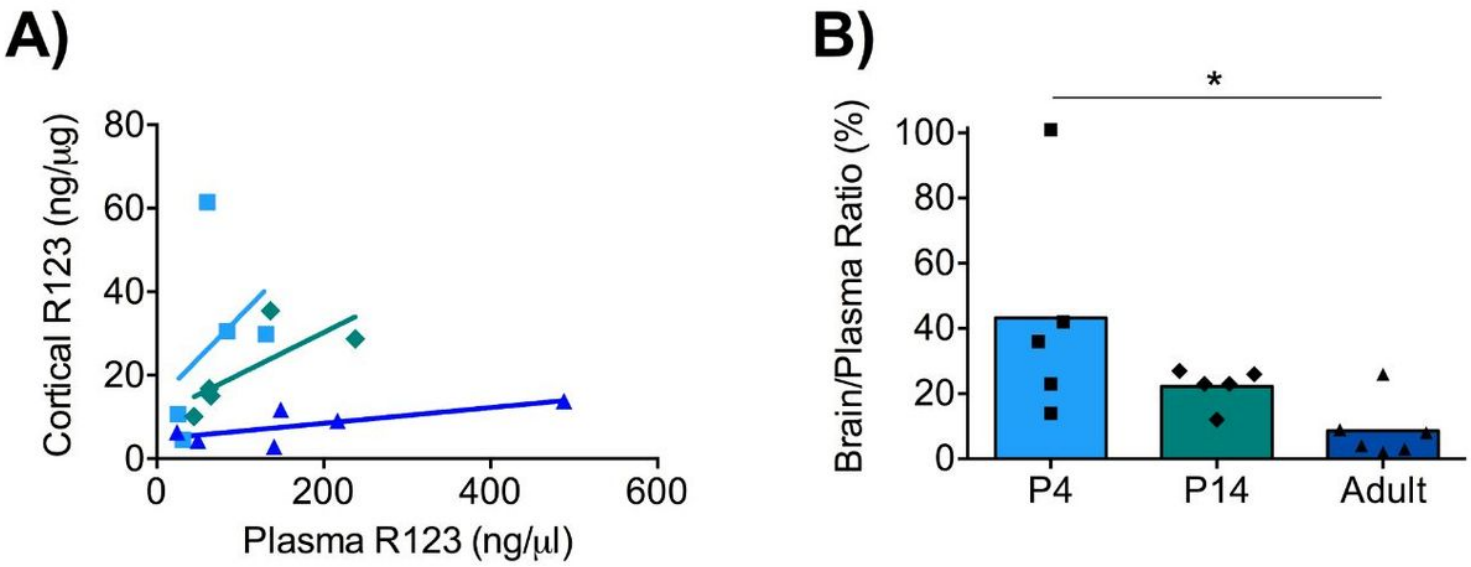

Figure 2

Transfer of R123 into the postnatal brain. A) The concentration of R123 in the brain cortices compared to the concentration in the plasma (blood). The three age groups investigated were P4 (light blue, squares), P14 (teal, diamonds) and adult (dark blue, triangles) rats. B) The concentration ratio between R123 in the brain and plasma. Graph A and B display data from the same rats. Significant differences between age groups are indicated: * $p<0.05$ (Kruskal-Wallis with Dunn's correction).

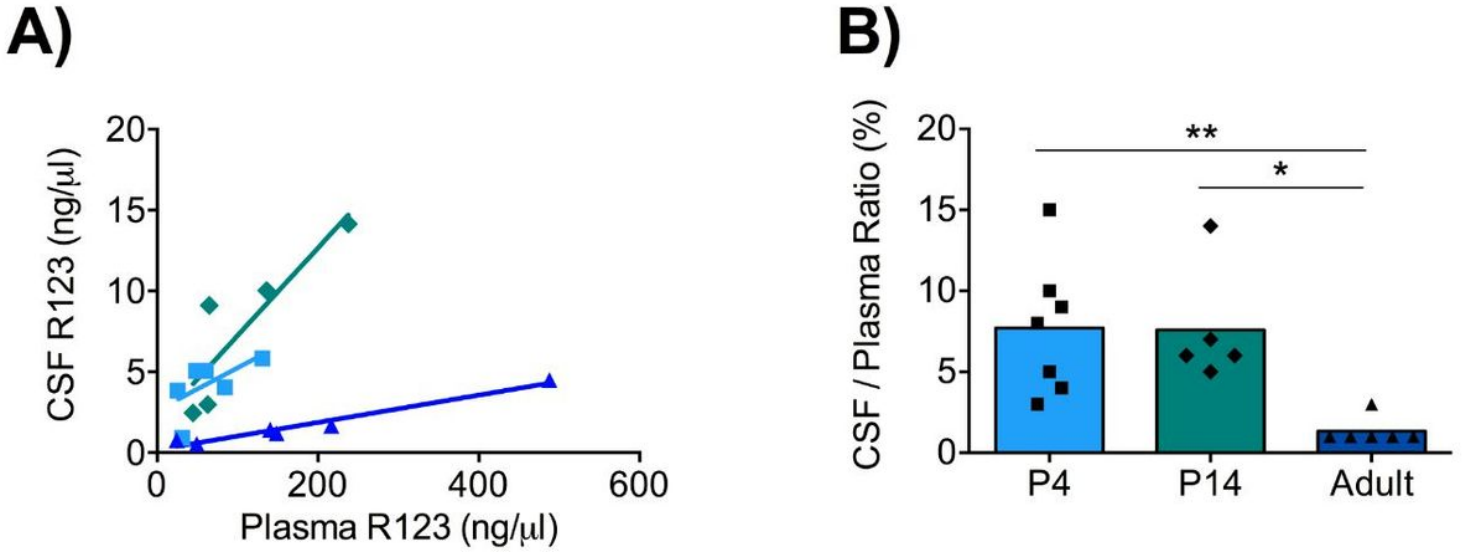

Figure 3

Transfer of R123 into the postnatal cerebrospinal fluid (CSF). A) The concentration of R123 in the CSF compared to the concentration in the plasma (blood). The three age groups investigated were P4 (light blue, squares), P14 (teal, diamonds) and adult (dark blue, triangles) rats. B) The concentration ratio between R123 in the brain and plasma. Graph A and B display data from the same rats. Significant differences between age groups are indicated: * $p<0.05$ and ** $p<0.01$ (Kruskal-Wallis with Dunn's correction).

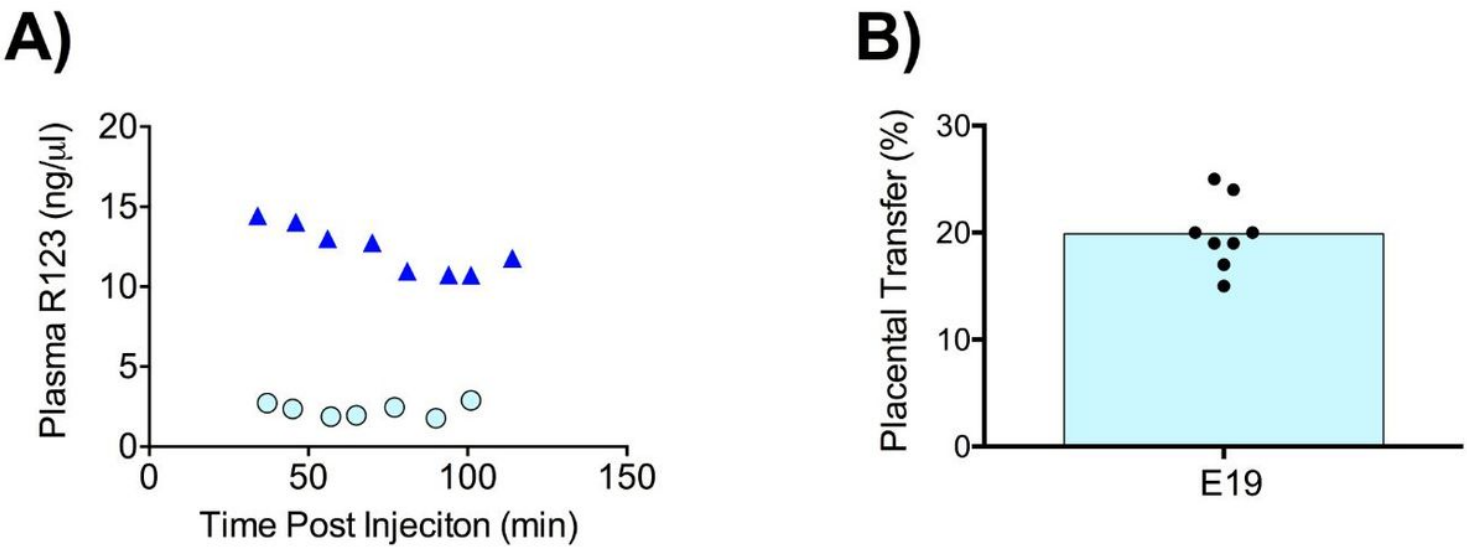

Figure 4

Transfer of R123 across the placenta. Concentration of R123 that reached the fetal blood (plasma) compared to the concentration in maternal plasma, following maternal injection at E19. A) R123 concentrations in maternal plasma (dark blue triangles) and fetal plasma (light blue circles) over time following maternal R123 injection. Each dot for maternal plasma indicates a serial sample over the course of experiment; each fetal sample indicates an individual pup. (B) Concentration ratios between fetal plasma and maternal plasma for all fetuses over the time course of 30-120min post maternal injection. Placental transfer (%) is a concentration ratio between R123 in fetal plasma compared to maternal plasma at the time of sampling.

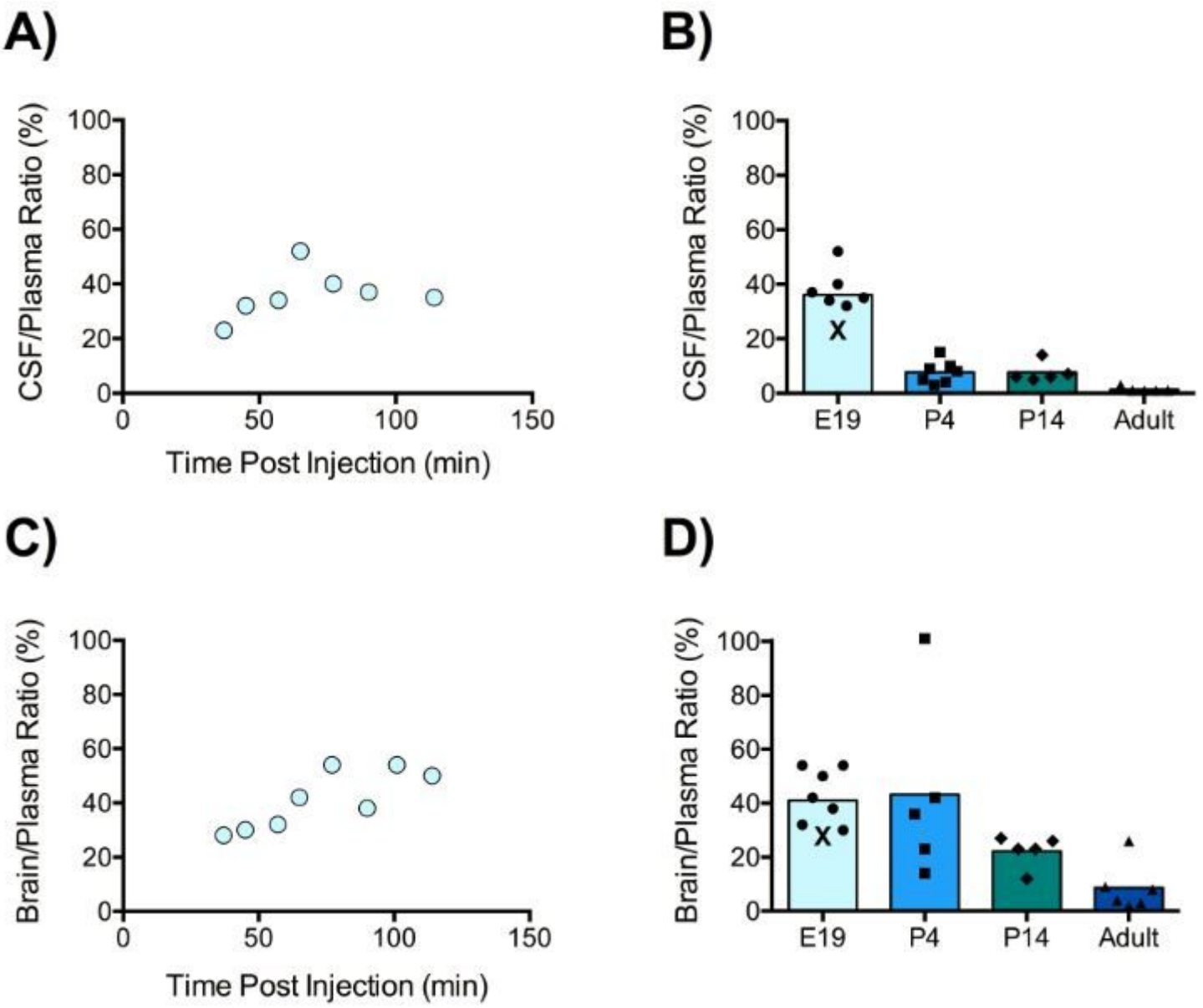

Figure 5

Transfer of R123 into the fetal (E19) brain and cerebrospinal fluid (CSF). Concentration ratios of R123 in the E19 CSF (A, B) or brain (C, D) compared to E19 plasma (blood) 30-120min post-maternal injection. The CSF/plasma (A) and brain/plasma (C) concentration ratios are shown compared to the time post-injection that each individual fetus was sampled (see Methods). For comparison the ratios at E19 are plotted with ratios obtained for P4, P14 and adult rats (B, D; data from Figures 2-3). The E19 concentration ratios taken closest to the 30min post-injection time-point of postnatal values are shown as X. Age is indicated on the X-axis (B,D).