pharmaceutics

MDPI

Supplementary Material:

Differential Blood–Brain Barrier Transport and Cell Uptake of Cyclic Peptides In Vivo and In Vitro

Table S1. Gradients used for cyclotide separation.

Program (steps)	Time (min)	Flow rate (mL/min)	%MPA	%MPB
		SFTI-1		
1	0.00	0.300	100.0	0.0
2	0.50	0.300	100.0	0.0
3	6.00	0.300	60.0	40.0
4	6.50	0.300	100.0	0.0
		Kalata B1		
1	0.00	0.300	90.0	10.0
2	2.00	0.300	45.0	55.0
3	2.50	0.300	90.0	10.0

Mobile phase A (MPA) was 90 % MilliQ-water, 10 % acetonitrile, and 0.05 % formic acid. Mobile phase B (MPB) was 10 % MilliQ-water, 90 % acetonitrile, and 0.05 % formic acid. Separation was performed on a Peptide CSH C18 column (50×2.1 mm; particle size, 1.7 µm) (Waters, MA, USA).

Table S2. Settings of TQS-micro mass spectrometer for MRM quantification of SFTI-1 and kalata B1.

Parameter	Unit	SFTI-1	Kalata B1
Capillary	kV	3.40	1.70
Cone	V	40.00	10.00
Source temperature	°C	150	150
Desolvation temperature	°C	500	500
Cone gas flow	L/h	20	0
Desolvation gas flow	L/h	1100	1000
Ion energy	-	3.00	–1.10

Table S3. Pharmacokinetic parameters used in simulation exercise.

Parameter	Unit	SFTI-1	Kalata B1
Systemic parameters			
Systemic clearance, *CL*	mL/min/kg	1.82	1.6
Systemic clearance, *CL*	L/h	0.0327	0.0288
Systemic clearance unbound, *CLu*	L/h	0.0307	0.0086
Apparent volume of distribution, V_d	mL/kg	191	322
Apparent volume of distribution, V_d	L	0.0573	0.0966
Apparent volume of distribution unbound, $V_{d,u}$	L	0.054	0.029
Fraction unbound in plasma, $f_{u,plasma}$	(Unitless)	0.936	0.299
Brain distribution parameters			
P_{app}	$\times 10^{-6}$ cm/s	2.62	3.19

P_{app} rat	$\times 10^{-6}$ mL/s	471.6[a]	574.2[a]
P_{app} rat	mL/s	0.00047	0.000572
CL_{in}	L/h	0.00170	0.00207
CL_{out}	L/h	0.01317	0.43155

Total brain-to-plasma concentration ratio, $K_{p,brain}$	(Unitless)	0.0618±0.0318	0.234±0.083
Unbound brain-to-plasma concentration ratio, $K_{p,uu,brain}$	(Unitless)	0.129	0.00479
Total steady-state plasma concentration, $C_{plasma,ss}$	nmol/L	400	1096
Total steady-state plasma concentration, $C_{plasma,ss}$	mg/L	0.6052	3.169632
Unbound steady-state plasma concentration, $C_{plasma,u,ss}$	mg/L	0.5664672	0.947719968
Unbound volume of distribution in brain, $V_{u,brain}$	mL/g brain	0.436	152
Unbound volume of distribution in brain, $V_{u,brain}$	L	0.00078	0.2736
Infusion rate	mg/h	0.03816	0.03816
Rat weight	kg	0.3	0.3
Brain weight	g	1.8	1.8
Surface area BBB*	cm^2	180	180
Molecular mass	Da	1513	2892

[a]Similar apparent permeability in human BBB cell culture model and rat endothelial cells was assumed.

* Hammarlund-Udenaes, M., Fridén, M., Syvänen, S. & Gupta, A. On the rate and extent of drug delivery to the brain. Pharm. Res. 25, 1737-1750 (2008).

Table S4. Permeability (Pe, 10^{-3} cm/min) to sodium fluorescein in in vitro BBB model in the presence or absence of SFTI-1 and kalata B1.

Treatment	Peptide concentration (nM)	Mean	SD
Control	0	0,46	0,12
SFT1	250	0,31	0,05
SFT1	500	0,32	0,02
kalata B1	500	0,50	0,10
kalata B1	1000	0,54	0,01
kalata B1	2000	0,47	0,07

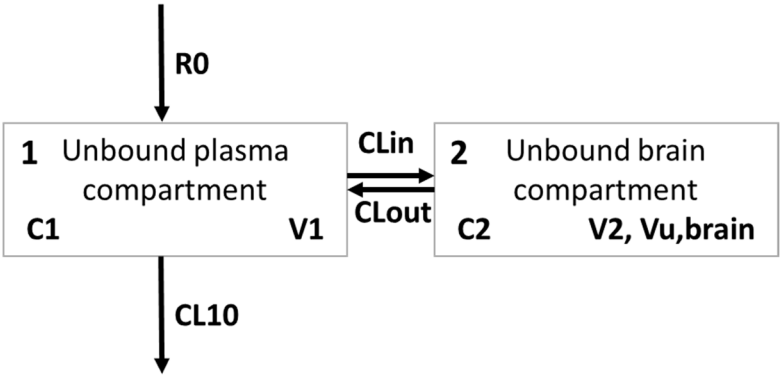

Figure S1. Model and code used for simulation of time concentration profiles for SFTI-1 and kalata B1.

1. SFTI-1

```
METHOD RK4
STARTTIME = 0
STOPTIME=24
DT = 0.02
d/dt(C1)=(R0-CL10*C1)/V1
d/dt(C2)=(CLin*C1-CLout*C2)/Vubrain
init C1 = 0.57; mg/L
init C2 = 0; mg/L
R0 = IF TIME <= INFSTOP THEN INFRATE ELSE 0
CL10 = 0.0307; L/h
CLin = 0.0016977; L/h
CLout = 0.01316; L/h
V1 = 0.054; L
Vubrain = 0.00078; L
INFRATE = 0.04; mg/h
INFSTOP = 4; h
```

2. Kalata B1

```
METHOD RK4
STARTTIME = 0
STOPTIME=24
DT = 0.02
d/dt(C1)=(R0-CL10*C1)/V1
d/dt(C2)=(CLin*C1-CLout*C2)/Vubrain
init C1 = 0.95; mg/L
init C2 = 0; mg/L
R0 = IF TIME <= INFSTOP THEN INFRATE ELSE 0
CL10 = 0.0086; L/h
CLin = 0.00206; L/h
CLout = 0.43; L/h
V1 = 0.0288 ; L
Vubrain = 0.274; L
INFRATE = 0.04; mg/h
INFSTOP = 4; h
```

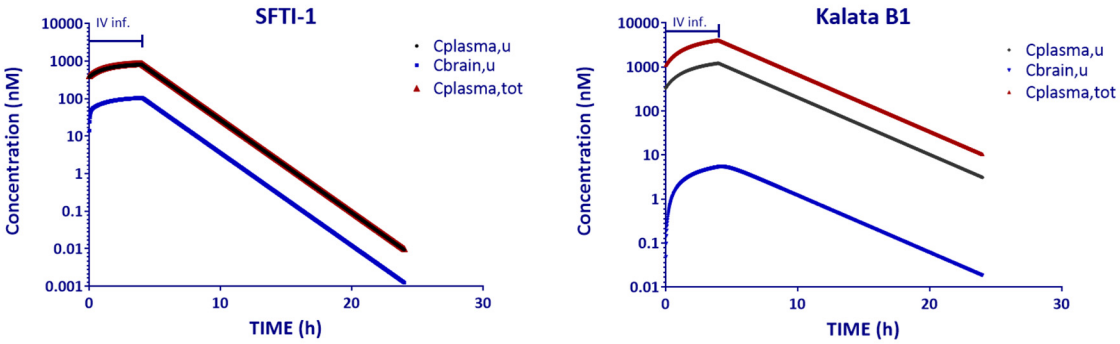

Figure S2. Simulation of total plasma (red), unbound plasma (black), and unbound brain (blue) concentrations (nM) of SFTI-1 and kalata B1 up to 24 h after and during a 4-hour constant-rate intravenous infusion (IV inf.) of the respective peptides. Because most SFTI-1 is unbound in plasma ($f_{u,plasma}$ is 0.936), the unbound and total plasma concentrations of SFTI-1 are overlapping. The initial total plasma concentrations were selected based on the achieved *in vivo* concentration in rats in the current study, i.e., 400 nM for SFTI-1 and 1000 nM for kalata B1. NB: for direct comparison between two cCPPs the concentrations are presented in nM.

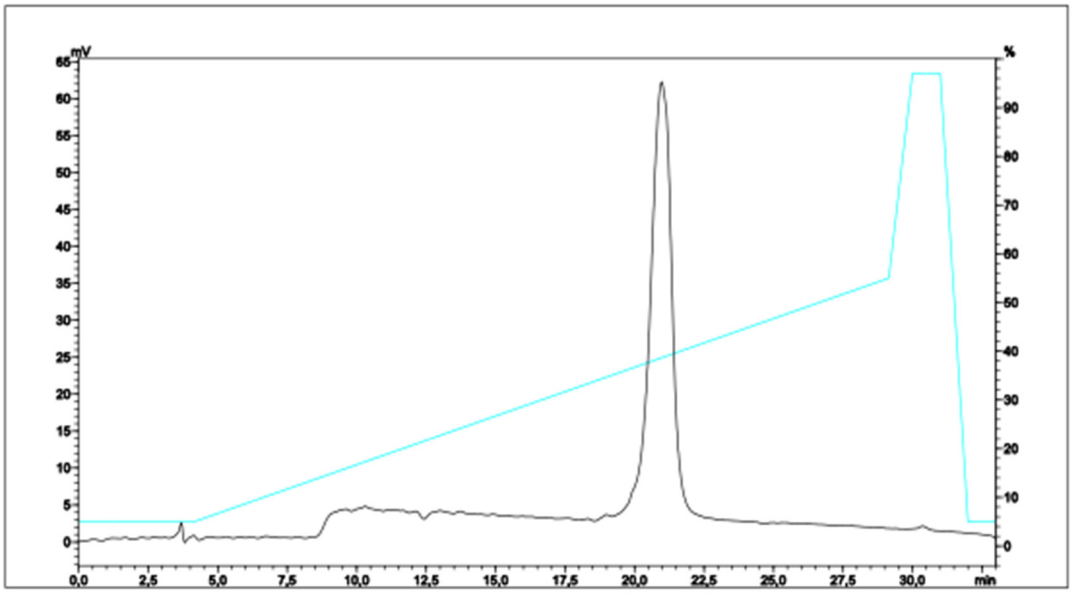

Figure S3. HPLC-UV chromatogram showing purity of SFTI-1, recorded at wavelength 215 nm. Gradient (in cyan) is the percentage of solvent B (0.045% TFA in ACN).