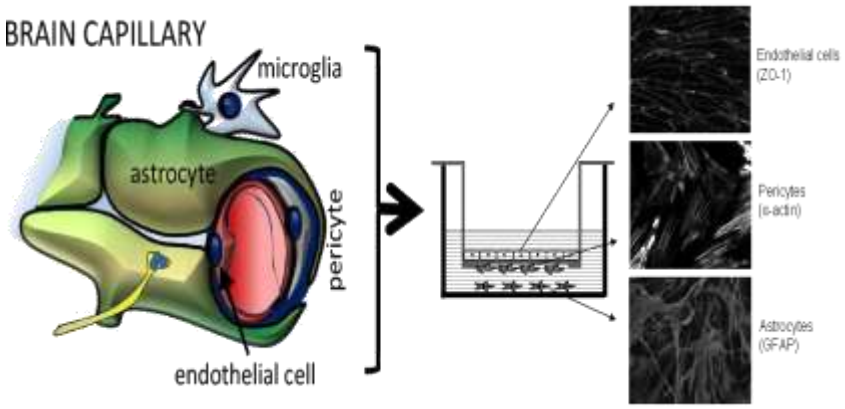
NEUROMICS

A Division of CA3 Biosciences, Inc.

3D Human Blood Brain Barrier Compound Penetration



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1. RESULT

1) Purpose

The objective of this study was to evaluate the BBB permeability of the test articles by 3D Human BBB model

2) Study conditions

This study was performed under GLP conditions. All work was performed with appropriate local health regulations and ethical approval. Two replicates were applied for each test compound.

3) Data Summary

Table 1. BBB Test Result

Compound	Test Conc. (μM)	Incubation Time (hour)	Mean P_e (nm/s)	Mean %Recovery	Permeability
Atenolol	10	4	<0.184*	<90.4	Low
Propranolol	10	4	52.1	98.5	High
Sulpiride	10	4	<0.184	<93.5	Low
Hydroxyzine Dihydrochloride	10	4	29.5	52.4	High
Hydrocortisone	10	4	9.17	87.6	Moderate
Cimetidine	10	4	<0.101	<93.4	Low
Propranolol hydrochloride	10	4	99.3	41.7	High
Atenolol	10	4	<0.184	<95.7	Low

Classification:
Low permeability: $P_e < 1.00$ nm/s
Moderate permeability: $1.00 < P_e < 10.0$ nm/s
High permeability: $P_e > 10.0$ nm/s

** The signal responses of Atenolol , Sulpiride, and Cimetidine in receiver samples were undetectable. For the convenience of calculation, 1/300 of the measured peak area ratio (PAR) of the relevant AD (A>B Donor) samples were used as the PAR values of Atenolol , Sulpiride, and Cimetidine in receiver samples.*

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2. MATERIALS

1) Preparation of PBS (100 mM phosphate, pH = 7.4 ± 0.05)

2.6 g KH_2PO4 and 18.5 g $K_2HPO_4{*}3H_2O$ were dissolved in 1000 mL of ultra-pure water, mixed thoroughly. The pH was adjusted to 7.40 ± 0.05, using either 1 M sodium hydroxide or 1M hydrochloric acid.

Table 2. Test Item Information

Compound ID	Lot#	M.W.	F.W.	Formula	Purity%
Sulpiride	BCCC9209	341.43	341.43	C15H23N3O4S	98
Hydroxyzine Dihydrochloride	BCBT3136	374.91	447.83	C21H27ClN2O2·2HCl	98
Hydrocortisone	SLCB9138	362.5	362.5	C21H30O5	98.0
Cimetidine	BCCC3082	252.34	252.34	C10H16N6S	98
Propranolol hydrochloride	BCCB3791	259.35	295.8	C16H21NO2·HCl	98
Atenolol	LRAC4829	266.34	266.34	C14H22N2O3	98

3. METHODS

1) Preparation of Donor Solution

a) For Sulpiride, 0.200 mM working solution was prepared by diluting 10.0 mM stock solution with Ethanol. For Hydrocortisone, 0.200 mM working solution was prepared by diluting 10.0 mM stock solution with DMSO. For others test compounds, 0.200 mM working solution was prepared by diluting 10.0 mM stock solution with H2O. For control compounds, 0.200 mM working solution was prepared by diluting 10.0 mM stock solution with DMSO.

b) 10 µM donor solution (5% DMSO) was prepared by diluting 20 µL of working solution with 380 µL PBS.

2) 150 µL of 10 µM donor solutions to each well of the donor plate. Duplicates were prepared.

3) 300 µL of PBS was added to each well of the plate.

4) The donor plate and acceptor plate were combined and incubated for 4h at room temperature with shaking at 300 rpm.

5) Preparation of T0 sample: 20 µL donor solution was transferred to new well followed by the addition of 250 µL PBS (DF: 13.5), 130 µL ACN (containing internal standard) as T0 sample.

6) Preparation of acceptor sample: The plate was removed from incubator. 270 µL solution was transferred from each acceptor well and mixed with 130 µL ACN (containing internal standard) as acceptor sample.

7) Preparation of donor sample: 20 µL solution was transferred from each donor well and mixed with 250 µL PBS (DF: 13.5), 130 µL ACN (containing internal standard) as donor sample.

8) Acceptor samples and donor samples were all analyzed by LC/MS/MS.

9) The equation used to determine permeability rates (P_e) was displayed as follow.

$$P_e = C \times (-\ln(1 - \frac{[drug]_{acceptor}}{[drug]_{equilibrium}})) \times 10^7, where C = (\frac{V_D \times V_A}{(V_D + V_A) \times Area \times time})$$

$[drug]_{equilibrium}=([drug]_{donor} \times V_D + [drug]_{acceptor} \times V_A)/(V_D+V_A)$

V_D = 0.15 mL; V_A = 0.30 mL; Area = 0.28 cm^2; time = 14400 s.

*$[drug]_{acceptor}$ = (Aa/Ai×DF)acceptor; $[drug]_{donor}$= (Aa/Ai*DF)donor;*

Aa/Ai: Peak area ratio of analyte and internal standard; DF: Dilution factor.

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