A Novel Three-Dimensional Glioma Blood Brain Barrier Model for High Throughput Testing of Tumoricidal Capability

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ABSTRACT

Brain cancer is one of the most difficult cancers to treat due to the brain's own defense system known as the blood brain barrier (BBB). The blood brain barrier, which is meant to protect the brain from potential toxins, often prevents conventional chemotherapeutics from reaching brain tumors[1]. Traditionally, high throughput testing of compound permeability through the BBB *in vitro* has been limited to assay of radio- or fluorophore-labeled compounds as they pass a cell monolayer growing on a permeable support system. Unfortunately, the labels themselves may impact the assay, and the ability to determine resulting tumor cytotoxicity must be studied independently. Here, we demonstrate a three dimensional (3D) model to study BBB transport as well as the resulting brain tumor cytotoxicity, by combining two commercially available products: Corning® 96 well spheroid microplates and the Corning HTS Transwell®-96 Tissue Culture System. Corning spheroid microplates are cell culture microplates with round well-bottom geometry coated with Corning Ultra-Low Attachment surface, enabling the formation of single multi-cellular tumor spheroids centered in each well in a highly reproducible manner. Corning HTS Transwells are permeable support systems commonly used for drug transport and migration/invasion studies. By replacing the standard flat-bottom Transwell receiver plate with a Corning spheroid microplate, we achieve the ability to study drug transport across the BBB and the resulting 3D glioma spheroid toxicity in an easy-to-use, 3D, high-throughput assay.

METHODS/MATERIALS

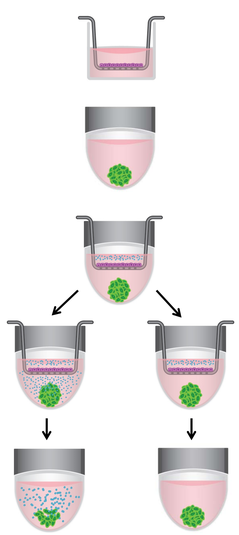
Blood brain barrier model
MDCKII/MDR1 cells were attained from Dr. Piet Borst (Netherlands Cancer Institute, Amsterdam, the Netherlands) and seeded into HTS 96-well Transwells (Corning Cat. No. 3391 or 3977) at 100,000 cells per cm^2 in 100 µL of Dulbecco's Modification of Eagle's Medium (DMEM) (Corning Cat. No. 10-013-CM) supplemented with 10% fetal bovine serum (FBS) (Corning Cat. No. 35-010-CV). They were cultured for 5 days with a medium exchange 24 hours prior to assay. Monolayer integrity was assessed via lucifer yellow permeability (Sigma Cat. No. L0144) and rhodamine 123 P-glycoprotein (Pgp) efflux (Sigma Cat. No. R8004). Immunostaining of MDCKII/MDR1 monolayers was performed in order to confirm presence of tight junction proteins ZO1 (Thermo Fisher Cat. No. 339188) and occludin (Thermo Fisher Cat. No. 331588) per manufacturer's protocol.

Gliomasphere formation
LN229 cells (ATCC® Cat. No. CRL-2611™) were routinely cultured in DMEM containing 10% FBS. Cells were harvested with Accutase® cell detachment solution (Corning Cat. No. 25-058-CI) and seeded into 96 well spheroid microplates at 1,000 cells per well for 24 hours prior to assay.

Blood brain barrier/gliomasohere model test
After 5 days of BBB formation medium from Transwells was aspirated and medium in the apical chamber was replaced with 250 µM cisplatin, piperlongumine or buffer which was matched to contain DMSO for piperlongumine. Inserts were then combined with 24 hour-old LN229 spheroids for 2 hours at 37ºC. After 2 hours of co-incubation inserts were removed and tested for barrier integrity via lucifer yellow. LN229 spheroids were cultured for two days and then assessed for viability with CellTiter GLO® 3D (Promega Cat. No. G9683).

Blood brain barrier/gliomasohere screen
After 5 days of BBB formation medium from Transwells was aspirated and medium in the apical chamber was replaced with 50 µL of compound from the Tocriscreen Kinase Inhibitor Toolbox (Tocris Cat. No 3514) or buffer. Inserts were then combined with 24 hour-old LN229 spheroids for 2 hours at 37ºC. After 2 hours of co-incubation, inserts were removed, tested for barrier integrity and LN229 spheroids were cultured and assayed as described previously.

Assay Schematic



Day 0: Seed MDCKII/MDR1 into 96 HTS Transwells

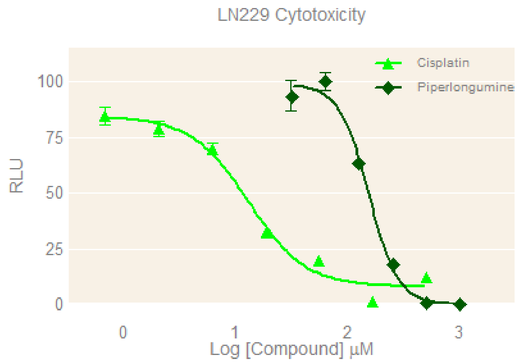
Day 4: Seed LN229 cells into Spheroid microplate

Day 5: Combine Transwell and Spheroid microplate and expose to drugs for 2 hours. After drug incubation remove Transwell and test for monolayer integrity. Culture spheroids for 2 additional days.

Day 7: Assay spheroids with Cell Titer Glow 3D

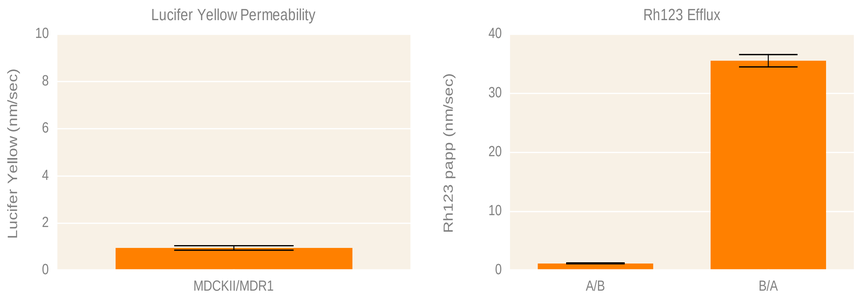
RESULTS

Spheroid Cytotoxicity

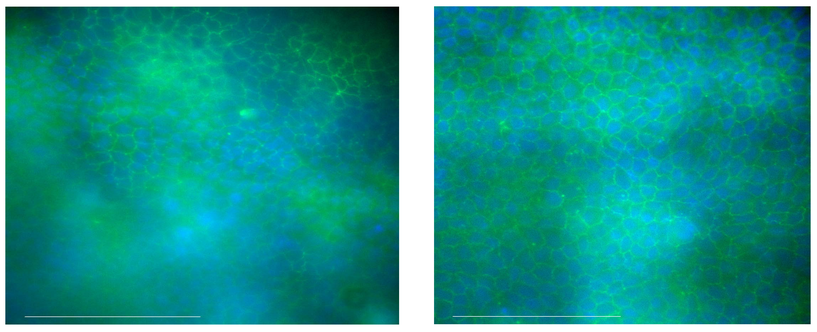


Dose dependent cytotoxicity of compounds
Dose dependent cytotoxicity of LN229 spheroids after 48 hours of direct culture with compounds. N = 12 wells per concentration form 2 independent studies.

Blood Brain Barrier Model

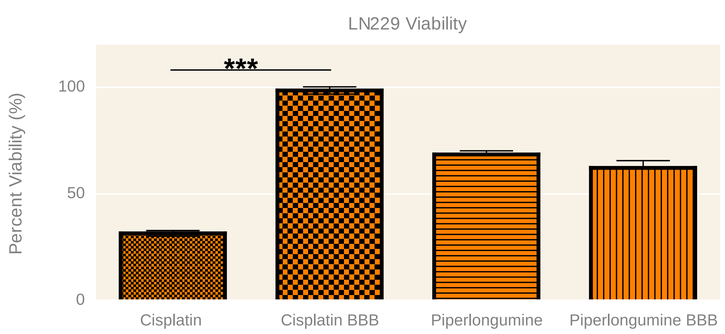


Low permeability and high P-glycoprotein 1 (Pgp) efflux activity
Lucifer yellow permeability and rhodamine 123 (Rh123) transport data after 5 days of culture on HTS 96-well Transwells. N = 120 from 3 independent studies.



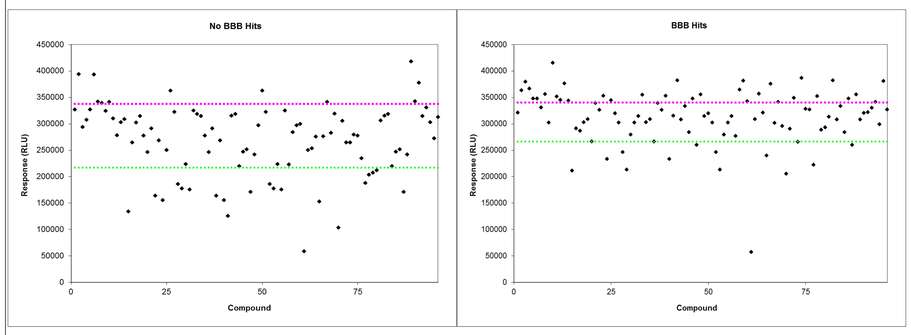
Expression of tight junction proteins. Representative photomicrographs of MDCK/IIMDR1 monolayers on HTS 96-well Transwells stained with ZO-1(left) and Occludin right) (green) and counterstained with Hoechst to show nuclei (blue). Isotype control image not shown (Thermo MA5-18167). Images taken at 40X with Thermo Scientific CX7. Scale is 100 µm.

Combined Model



LN229 Cytotoxicity with or without blood brain barrier surrogate
Percent viability of LN229 spheroids 48 hours post 2 hour 250 µM drug exposure through Transwells with or without a BBB. Viability was assessed by normalizing buffer controls to 100% viability. Data shown as the average of 3 independent studies, N = 30 with 1-way ANOVA with Bonferroni's post test. *** = p<0.0001

Representative Drug Screen



Representative Screen
Representative screen from Tocriscreen Kinase Inhibitor Toolbox showing hits found with and without HTS 96-well Transwells containing MDCKII/MDR1 BBB. The pink line is average buffer response and the green line represents 3 sigma below buffer response.

Summary of Hits

	1	2	3	4	5	6	7	8	9	10	11	12
A	Buffer	0414 AG 490	0431 ML 9 hydrochloride	3439 NH 125	0541 Fasudil hydrochloride	0741 GF 109203X	1110 Genistein	1130 LY 294002 hydrochloride	1144 U0126	1213 PD 98059	1254 Y-27632 dihydrochloride	Buffer
B	Buffer	1264 SB 202190	1284 Olomoucine	1300 LFM-A13	1321 ZM 336372	1366 ZM 449829	1367 ZM 39923 hydrochloride	1381 GW 5074	1397 PP 1	1402 SB 203580 hydrochloride	1405 (-)-Terreic acid	Buffer
C	Buffer	1407 PP 2	1459 SU 4312	1496 SP 600125	1580 Purvalanol A	1581 Purvalanol B	3544 KU 55933	1614 SB 431542	1616 SB 216763	1617 SB 415286	1777 Arctigenin	Buffer
D	Buffer	1937 NSC 693868	1962 SB 239063	1969 SL 327	2002 Ro 31-8220 mesylate	2072 Aminopurvalanol A	2151 API-2	2238 GW 441756	2239 GW 583340 dihydrochloride	2272 Ro 08-2750	2275 TBB	Buffer
E	Buffer	2291 1,2,3,4,5,6-Hexabromocyclohexane	2415 HA 1100 hydrochloride	2416 BIBX 1382 dihydrochloride	2442 CGP 53353	2457 Arcyriaflavin A	2458 ZM 447439	2471 ER 27319 maleate	2475 ZM 323881 hydrochloride	2499 ZM 306416 hydrochloride	2539 IKK 16	Buffer
F	Buffer	2542 Ki 8751	2558 10-DEBC hydrochloride	2559 TPCA-1	2560 SB 218078	2591 TCS 359	2605 PD 198306	2609 Ryuvidine	2611 IMD 0354	2639 CGK 733	2693 PHA 665752	Buffer
G	Buffer	2694 PD 407824	2718 LY 364947	2731 CGP 57380	2768 PQ 401	2814 PI 828	2828 NU 7026	2902 D 4476	2908 EO 1428	2910 H 89 dihydrochloride	2926 FPA 124	Buffer
H	Buffer	2977 GW 843682X	3000 Iressa	3037 SU 5416	3063 1-Naphthyl PP1	3572 GSK 650394	3194 BIO	3269 SD 208	3271 Compound 401	3314 BI 78D3	3318 SC 514	Buffer

Screen Summary
Compilation of hits discovered with or without HTS 96-well Transwells containing MDCKII/MDR1 BBB. Inserts that exhibited higher than 10 nm/sec lucifer yellow permeability after compound exposure were discarded from analysis since monolayer was deemed compromised. Hits were considered if they were 3 sigma below buffer response in at least 2 of 3 independent screens. Blue boxes are hits only found without BBB. Purple boxes were hits found with and without BBB. Blue boxes were only found when BBB was not present.

SUMMARY/CONCLUSIONS

- Corning 96-well spheroid microplates allow for the formation of consistent sized, single spheroids in each well that are ideally suited for drug screens.
- MDCKII/MDR1 cells can successfully form a barrier on Corning HTS 96-well Transwells and are capable of demonstrating low LY permeability, high Pgp efflux activity and tight junction formation.
- The combination of Corning spheroid microplates and the HTS 96-well Transwells allow for a novel 3D model that can differentiate between compounds that can pass the BBB and those that can not while also assessing the resulting gliomasphere cytotoxicity.

REFERENCES

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2. Kim, Tae Hyong, et al. "Piperlongumine treatment inactivates peroxiredoxin 4, exacerbates endoplasmic reticulum stress, and preferentially kills high-grade glioma cells." Neuro-Oncology 16.10 (2014): 1354-1364.
3. Zhang, Lin, et al. "Influence of puerarin, paeoniflorin, and menthol on structure and barrier function of tight junctions in MDCK and MDCK-MDR1 Cells." Journal of Traditional Chinese Medical Sciences 2.2 (2015): 111-119.