Case Study

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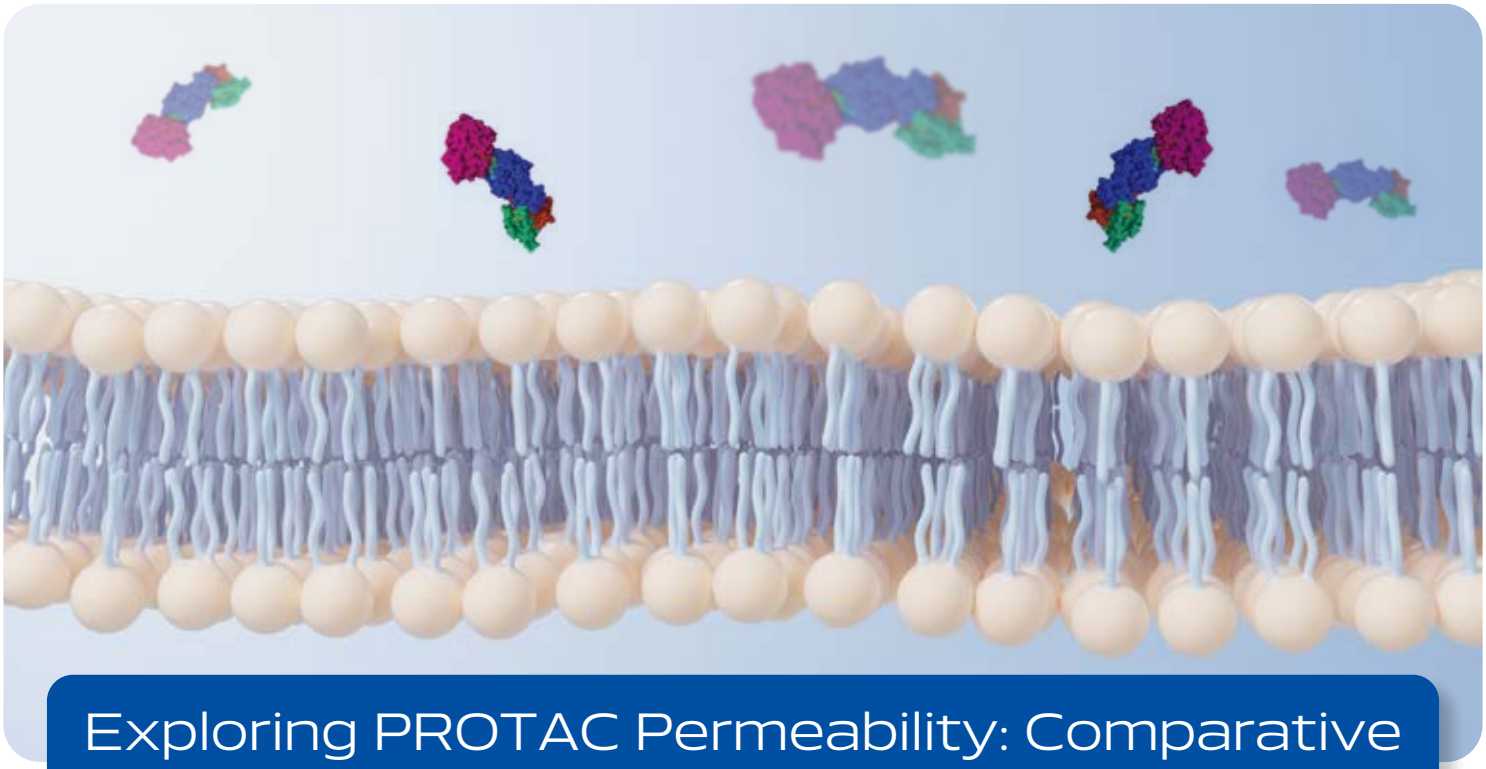

Exploring PROTAC Permeability: Comparative Insights Across Different Cell Lines

Overview

The permeability of Proteolysis Targeting Chimeras (PROTACs) is a crucial determinant for their bioavailability and therapeutic efficacy. PROTACs are bifunctional molecules that induce targeted protein degradation by binding both a protein of interest and an E3 ligase. However, their larger size and complex structure often challenge their ability to efficiently cross biological membranes, making permeability a key consideration in drug discovery and development.

Passive permeability, which allows compounds to diffuse across membranes without the help of active transporters, is crucial for PROTACs. However, factors such as high molecular weight, polar surface area, and exposed hydrogen bond donors frequently hinder this process.

Unlike traditional small molecules that follow Lipinski's Rule of 5 for predicting permeability and oral bioavailability, PROTACs often exceed these guidelines, making their transport across biological barriers challenging. Therefore, choosing the right cell models to evaluate their permeability is essential.

Aragen's Approach

In this study, we evaluated the **permeability characteristics** of three PROTACs—**ARV-771 (ALS-010), ARV-110 (ALS-011),** and **KT-474 (ALS-012)** along with three quality control (QC) compounds: Propranolol (highly permeable), Atenolol (low permeable), and Digoxin [a P-glycoprotein (P-gp) substrate] - using three different cell lines:

- **LLC-PK1 cells:** Used to assess passive permeability and transport properties.
- **MDR1 MDCK-II cells:** Employed to study drug efflux via the P-gp efflux pump.
- **Caco-2 cells:** A gold standard for evaluating intestinal permeability, relevant for oral bioavailability studies.

Permeability assays were performed using Millicell 96-transwell plate, with cells cultured under standard conditions. The following parameters were measured:

- **Apical-to-basal (P_{app}) and basal-to-apical (P_{app})** permeability to assess the transport across the cell monolayer.
- **Efflux Ratio** to measure the active transport of compounds.
- **% Recovery** in both directions (apical-to-basal and basal-to-apical) to evaluate the compound's retention and transport efficiency.
- **Transepithelial Electrical Resistance (TEER)** was measured to assess the integrity of the cell monolayer and ensure that proper barriers were formed.
- **Luciferase Yellow Assay** was performed post-incubation of the permeability assay to assess the cell monolayer integrity.

Cell Culture Parameters

Cell Line	Cell Density/Well	Days of Culture	TEER (Ω/cm^2)
LLC-PK1	25,000	4	>90
MDR1 MDCK-II	25,000	7	>65
Caco-2	12,000	21	>230

Outcomes

The study compared the permeability of PROTACs across three cell lines—LLC-PK1, MDR1 MDCK-II, and Caco-2—with the hypothesis that LLC-PK1 offers advantages over the other two models. The findings highlight differences in permeability, efflux ratios (Table 1), and recovery rates (Table 2), providing insights into the suitability of each cell line for assessing PROTAC transport and absorption.

Permeability Profiles and Efflux Ratios

- LLC-PK1 exhibited improved permeability with lower efflux ratios compared to MDR1 MDCK-II and Caco-2, making it suitable for passive permeability studies.
- ARV-771 exhibited extremely high efflux ratios in Caco-2 cells (87.62 ± 1.51), indicating strong transporter activity.
- KT-474 showed high efflux in MDR1 MDCK-II cells (57.54 ± 2.22), suggesting significant P-gp-mediated transport.
- ARV-110 had negligible permeability in Caco-2 cells (P_{app} = 0), highlighting challenges with this model for certain PROTACs.

Table 1: Comparison of permeability and efflux ratios for PROTACs (ARV-771, ARV-110, and KT-474) and QC compounds Propranolol, Atenolol, Digoxin) across LLC-PK1, MDR1 MDCK-II, and Caco-2 cell lines.

Compound	Cell Line	Apical to Basal (Papp, x 10^{-6} cm/s)	Basal to Apical (Papp, x 10^{-6} cm/s)	Efflux Ratio
ARV-771 (ALS-010)	LLC-PK1	0.28 ± 0.00	2.29 ± 2.84	15.27 ± 0.10
	MDR1 MDCK-II	0.12 ± 0.05	0.96 ± 0.20	7.34 ± 2.22
	Caco-2	0.14 ± 0.01	12.34 ± 0.82	87.62 ± 1.51
ARV-110 (ALS-011)	LLC-PK1	0.03 ± 0.02	0.04 ± 0.02	2.09 ± 1.29
	MDR1 MDCK-II	0.02 ± 0.03	0.17 ± 0.01	4.57 ± 0.53
	Caco-2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
KT-474 (ALS-012)	LLC-PK1	0.56 ± 0.13	1.71 ± 1.51	6.22 ± 0.27
	MDR1 MDCK-II	0.21 ± 0.06	11.59 ± 0.49	57.54 ± 2.22
	Caco-2	0.31 ± 0.01	0.79 ± 0.12	2.22 ± 2.55

QC Compound	Cell Line	Apical to Basal (Papp, x 10^{-6} cm/s)	Basal to Apical (Papp, x 10^{-6} cm/s)	Efflux Ratio
Digoxin	LLC-PK1	2.40 ± 0.31	6.57 ± 1.11	3.08 ± 0.46
	MDR1 MDCK-II	0.49 ± 0.03	14.60 ± 4.30	35.43 ± 0.99
	Caco-2	1.76 ± 0.17	28.13 ± 1.36	16.16 ± 2.27
Propranolol	LLC-PK1	34.20 ± 1.84	33.51 ± 2.64	1.00 ± 0.15
	MDR1 MDCK-II	27.74 ± 0.50	34.78 ± 3.60	1.26 ± 0.15
	Caco-2	19.55 ± 0.74	21.76 ± 0.04	1.12 ± 0.05
Atenolol	LLC-PK1	0.93 ± 0.20	0.47 ± 0.01	0.53 ± 0.10
	MDR1 MDCK-II	0.74 ± 0.60	0.68 ± 0.39	1.07 ± 0.34
	Caco-2	0.32 ± 0.06	0.31 ± 0.00	1.01 ± 0.16

Recovery Rates

- LLC-PK1 demonstrated consistent recovery rates (~65–82%)
- Caco-2 cells showed lower recovery rates (~48–82%) i.e. less than the acceptable limit (<65%) suggesting potential metabolism or cellular entrapment or adsorption issues.

Table 2: Percentage Recovery (apical to basal and basal to apical) of PROTACs (ARV-771, ARV-110, and KT-474) and QC compounds Propranolol, Atenolol, Digoxin) across LLC-PK1, MDR1 MDCK-II, and Caco-2 cell lines.

Compound	Cell Line	% Recovery (Apical to Basal)	% Recovery
ARV-771 (ALS-010)	LLC-PK1	72.42 ± 1.11	79.98 ± 3.69
	MDR1 MDCK-II	71.07 ± 1.88	63.78 ± 5.13
	Caco-2	57.05 ± 1.82	60.57 ± 4.63
ARV-110 (ALS-011)	LLC-PK1	76.54 ± 1.22	82.97 ± 4.04
	MDR1 MDCK-II	82.50 ± 4.49	84.48 ± 0.67
	Caco-2	79.86 ± 0.76	82.52 ± 6.23
KT-474 (ALS-012)	LLC-PK1	20.82 ± 3.61	65.92 ± 0.47
	MDR1 MDCK-II	34.48 ± 7.86	48.14 ± 9.36
	Caco-2	8.56 ± 4.19	48.60 ± 7.08

QC Compound	Cell Line	% Recovery (Apical to Basal)	% Recovery (Basal to Apical)
Digoxin	LLC-PK1	78.58 ± 4.82	88.52 ± 4.61
	MDR1 MDCK-II	85.81 ± 7.18	86.73 ± 6.81
	Caco-2	82.94 ± 1.50	99.05 ± 5.80
Propranolol	LLC-PK1	88.65 ± 3.60	87.94 ± 1.85
	MDR1 MDCK-II	82.23 ± 10.28	85.76 ± 4.53
	Caco-2	81.83 ± 5.91	88.38 ± 3.20
Atenolol	LLC-PK1	89.24 ± 2.80	94.55 ± 0.83
	MDR1 MDCK-II	85.84 ± 5.45	87.56 ± 0.01
	Caco-2	92.45 ± 3.40	93.45 ± 0.35

Key Takeaways

Advantages of LLC-PK1 Cell Line

- LLC-PK1 provides simpler handling and faster throughput compared to Caco-2 cells, which require extended culture times (21 days) for differentiation.
- Lower efflux activity in LLC-PK1 reduces transporter interference, making it ideal for studying passive permeability of PROTACs.

Utility of MDR1 MDCK-II Cell Line

- MDR1 MDCK-II is highly effective for probing transporter-mediated efflux due to its overexpression of P-gp.
- It is particularly useful for investigating drug-drug interactions involving efflux transporters.

Challenges with Caco-2 Cell Line

- While physiologically relevant, Caco-2 cells exhibit high efflux activity and interference of transporters, complicating permeability assessments for certain compounds like PROTACs.
- Their extended culture time limits their utility in high-throughput screening.

PROTAC-Specific Insights

- High efflux ratios observed in Caco-2 and MDR1 MDCK-II suggest that PROTACs are substrates for efflux transporters like P-gp.
- LLC-PK1's reduced transporter activity provides a clearer assessment of passive permeability.

Conclusion

The case study highlights that:

- LLC-PK1 cell line offers distinct advantages over MDR1 MDCK-II and Caco-2 cell lines for evaluating PROTAC permeability, particularly when passive diffusion is the primary focus.
- Ease of handling, lower transporter interference, and consistent recovery rates of LLC-PK1 cell line makes it a valuable tool for initial screening of PROTACs.
- In contrast, MDR1 MDCK-II excels in studying transporter-mediated efflux due to its high P-gp expression, while Caco-2 provides physiological relevance by mimicking human intestinal absorption, their limitations—high efflux ratios and extended culture times—make them less practical for initial permeability screening of PROTACs.
- Combining data from all three models provides a comprehensive understanding of transport mechanisms but positions LLC-PK1 as the preferred model for early-stage studies.

Why Aragen?

Aragen Life Sciences provides comprehensive, reliable, and efficient DMPK services tailored to meet drug discovery needs. We offer:

- **Expertise in PROTACs and Novel Modalities:** Specialized in evaluating challenging drug modalities like PROTACs, peptides, and oligonucleotides.
- **Comprehensive DMPK Solutions:** From exploratory studies to IND-enabling packages, we provide end-to-end support for ADME profiling, PK/PD correlation, and transporter interaction studies.
- **Rapid Turnaround Time:** High-quality data delivered within 5–7 working days—among the fastest in the industry.
- **Advanced Technologies:** Cutting-edge tools and AAALAC-accredited vivarium ensure precise and reliable results.
- **Proven Track Record:** Conducts over 12,000 ADME studies and 300,000 bioanalyses monthly, demonstrating efficiency and scalability.
- **Consultative Approach:** Works collaboratively with clients from study design to data interpretation, ensuring alignment with project goals.
- **Cost-Effective Solutions:** Offers competitive pricing without compromising quality, making it accessible for companies of all sizes.

Let's begin the Conversation

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