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In Vitro Permeability and Metabolic Biotransformation of Oral β-Lactamase Inhibitor VNRX-7145 Across Species

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Background

VNRX-7145 is a novel, orally bioavailable, cyclic boronate β-lactamase inhibitor (BLI) in development in combination with ceftibuten as an oral treatment for infections caused by serine-β-lactamase-producing Enterobacteriaceae. *In vivo*, VNRX-7145 undergoes biotransformation to the active BLI, VNRX-5236, that covalently and reversibly binds the active site serine of Ambler Class A, C and D β-lactamases[1]. Here, the bidirectional permeability through Caco-2 monolayers was investigated to determine the potential for human absorption. In addition, rates of metabolic transformation along with metabolite profiling across pre-clinical species and humans were determined.

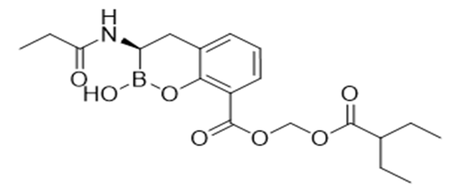
Methods

Caco-2 Assay: Caco-2 cell (C2BBe1) monolayers were dosed with 5 μM of VNRX-7145 on the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37°C with 5% CO_2 in a humidified incubator. Samples were taken from the donor and receiver chambers at 120 minutes. All samples were assayed by LC-MS/MS using EI for both VNRX-7145 and VNRX-5236.

Metabolic Stability: VNRX-7145 along with controls were incubated in intestinal S9, liver S9, and plasma of various species at 37°C. In the cases of intestinal S9 and liver S9, the incubations were done both in the presence and absence of NADPH to examine involvement of cytochrome P450 in the metabolism of VNRX-7145. Aliquots were removed at indicated times, treated with internal standard containing acetonitrile to precipitate protein, and evaporated to dryness. Samples were reconstituted in 0.1% formic acid in water and quantified by UPLC-MS/MS for VNRX-7145 and VNRX-5236.

Metabolite Profiling: VNRX-7145 was added to hepatocyte incubations prepared from cryopreserved hepatocytes of various species at 37°C. An aliquot of each incubation was taken at 4 hours, quenched with acetonitrile, centrifuged, and the resulting supernatants analyzed by UPLC-HRMS and UPLC-HRMS/MS.

Structure of VNRX-7145



Caco-2 Assay

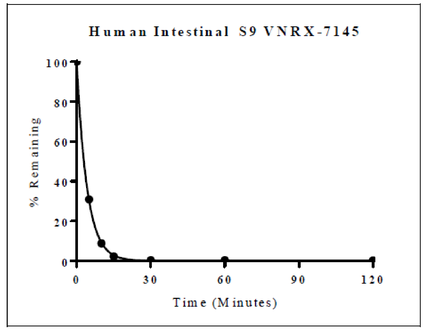
VNRX-7145: Bidirectional Permeability through Caco-2 Monolayers

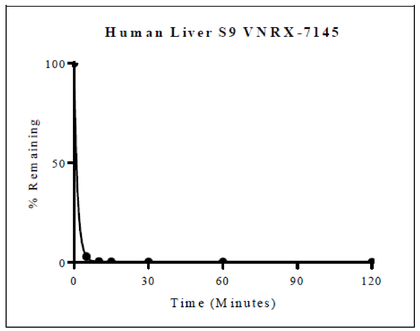
Direction	Recovery	P_{app} (10^{-6} cm/s)			Efflux Ratio[a]	Abs. Potential Classification[b]
		Run #1	Run #2	AVG		
A-to-B	90	8.25	10.2	9.22	3.2	**High**
B-to-A	98	28.3	30.5	29.4		

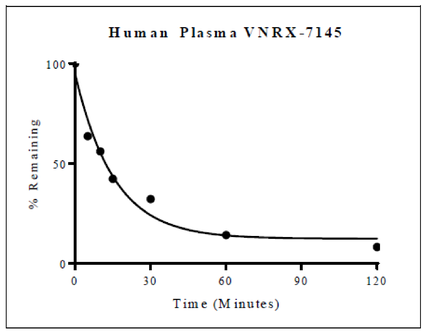
[a] Efflux ratio (ER) defined as P_{app} (B-to-A)/P_{app} (A-to-B)
[b] Absorption Potential Classification: P_{app} (A-to-B) < 1.0 x 10^{-6} cm/s: Low
P_{app} (A-to-B) > 1.0 x 10^{-6} cm/s: High

Metabolism of VNRX-7145 in Mouse, Rat, Dog, Monkey, and Human Intestinal S9, Liver S9, and Plasma

Species	Intestinal S9 Half-life (min)		Liver S9 Half-life (min)		Plasma Half-life (min)
	+NADPH	-NADPH	+NADPH	-NADPH	
CD-1® Mouse	1.1	1.1	1.2	1.3	4.6
Sprague-Dawley® Rat	3.9	4.0	2.3	2.8	1.6
Beagle Dog	49.0	119.4	0.5	0.7	43.9
Cynomolgus Monkey	11.2	10.1	0.8	0.9	22.0
Human	**2.9**	**3.3**	**1.0**	**1.1**	**10.7**

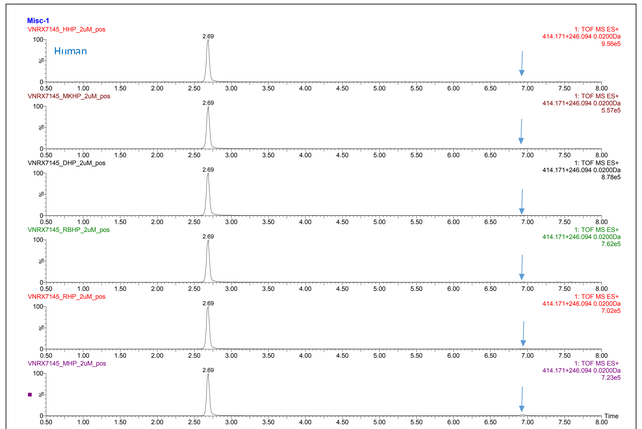






- Significant cleavage of VNRX-7145 to active BLI VNRX-5236 occurred in intestinal S9 incubations across species with the exception of beagle dog. No differences were observed in the presence or absence of NADPH.
- Liver S9 incubations also resulted in significant cleavage of VNRX-7145 across all species with no differences in the presence or absence of NADPH.
- The half-life of VNRX-7145 in human plasma was short and similar to the rodent species with both dog and monkey having longer half-lives.
- *In vivo* oral pharmacokinetic experiments confirmed that VNRX-7145 is highly absorbed and undergoes extensive and rapid cleavage to VNRX-5236 based on circulating levels[2].

Metabolite Profiling



After hepatocyte incubation, VNRX-5236 (active drug) was the only metabolite observed with little to no remaining VNRX-7145 observed at both concentrations (2 and 20 μM) tested across all species.

Conclusions

- VNRX-5236 (active BLI) was the only observed metabolite in metabolic profiling of VNRX-7145.
- Significant cleavage of the prodrug (VNRX-7145) to the active BLI (VNRX-5236) was observed in intestinal S9, liver S9, and plasma in all species tested.
- VNRX-7145 demonstrated high absorption potential through Caco-2 monolayers which, along with the rapid transformation to the active BLI and favorable metabolic stability, suggests that VNRX-7145 could have clinically relevant oral bioavailability in humans.

References

1. Meyers, C. et al. 2019. Ceftibuten/VNRX-7145, an orally bioavailable β -lactam/ β-lactamase inhibitor combination active against serine- β -lactamase-producing Enterobacteriaceae. ECCMID poster #1182
2. Pevear, D.C. et al. 2019. Oral Bioavailability of Novel β-Lactamase Inhibitor VNRX-7145 in Rats, Dogs, and Non-Human Primates. ASM poster #AAR-728

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