GUT BACTERIAL UREASE INHIBITION BY VS-02 AS A POTENTIAL TREATMENT TO REDUCE HYPERAMMONEMIA AND PROTECT FROM HEPATIC ENCEPHALOPATHY (HE) IN CIRRHOSIS

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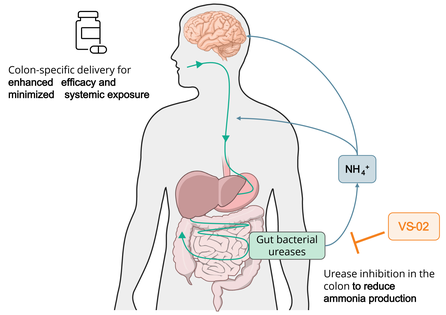
BACKGROUND & AIM

- Gut bacterial ureases, which convert urea into ammonia, contribute to systemic ammonia levels and thus represent a promising therapeutic target for reducing hyperammonemia and alleviating hepatic encephalopathy (HE)
- Hydroxamic acids (HAs) are potent urease inhibitors that have shown beneficial effects in preclinical models and patients with liver disease. Among them, acetohydroxamic acid (AHA)[1-3], octanohydroxamic acid (OHA)[4,5], and nicotinohydroxamic acid[6] are the only HAs that have been clinically tested in liver disease patients, primarily in studies conducted between the 1960s and 1990s. Despite encouraging results, none of these compounds advanced in further development for HE, possibly due to insufficient potency or a failure to reach effective concentrations in the colon, the main site of bacterial urease activity
- VS-02 is a hydroxamic acid derivative under development for the treatment of HE, formulated for colon-targeted delivery to enhance local inhibitor concentration at the site of ammonia production while minimizing systemic exposure



The aim of this study was

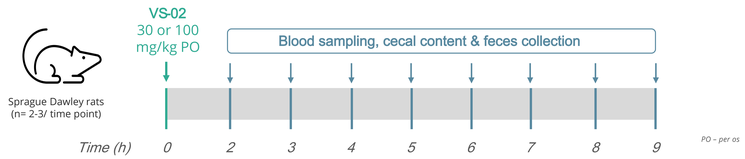
- **to evaluate the efficacy of VS-02 in reducing ammonia *ex vivo*, in comparison to other hydroxamic acids (AHA and OHA)**
- **to characterize the pharmacokinetic (PK) profile of preclinical formulation of VS-02 following oral administration to identify the dose level achieving effective concentrations in the cecum, a primary site of bacterial abundance in the rat gut**

METHODS

Ex vivo urease activity assay in rat cecal content

Urease inhibitory activity of HAs was evaluated in pooled cecal content of male Sprague Dawley rats (n=3), diluted to 5% w/v with 200 mM KH_2PO_4 (pH 6.8). After low-speed centrifugation to remove debris, bacteria were incubated with 100 mM urea and an inhibitor for 30 min at 37 °C. Ammonia levels were measured before (T0) and after incubation (T30') using a colorimetric urease activity assay kit (abcam, ab204697)

PK study in rats



PK of VS-02 was studied in healthy male Sprague Dawley rats. Two groups of rats (n=24/group) received single oral doses of 30 mg/kg or 100 mg/kg VS-02 *via* gavage

At pre-defined time points following dosing, rats were euthanized, and plasma, cecal content, and feces were collected for quantification of VS-02 by the LC-MS/MS system (SCIEX Triple Quad 6500+). PK parameters were calculated by non-compartmental analysis using Phoenix WinNonlin software (version 8.5.1.3)

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Illustration were obtained from NIAID NIH BioArt Source

RESULTS

VS-02 DEMONSTRATES SUPERIOR UREASE INHIBITORY ACTIVITY COMPARED TO REFERENCE HYDROXAMIC ACIDS

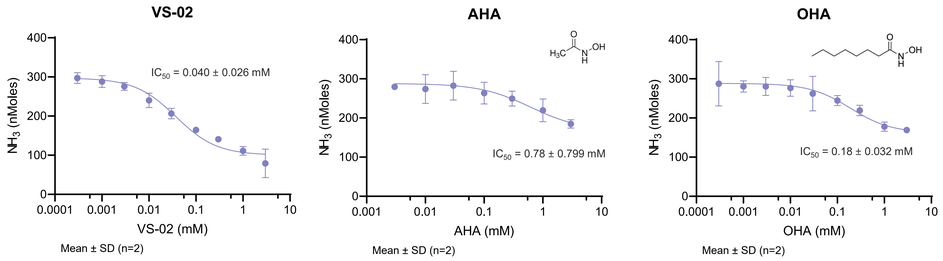

Figure 1: Dose-response curves for VS-02, AHA and OHA

→ **VS-02 demonstrated superior potency compared to AHA and OHA**

→ **To achieve ca. 70-90% of maximum inhibition (E_{max}) of urease by VS-02, a concentration range of 100 – 500 µM should be targeted at the site of action**

PRECLINICAL FORMULATION OF VS-02 LEADS TO HIGH LOCAL EXPOSURE IN THE CECUM OF HEALTHY RATS

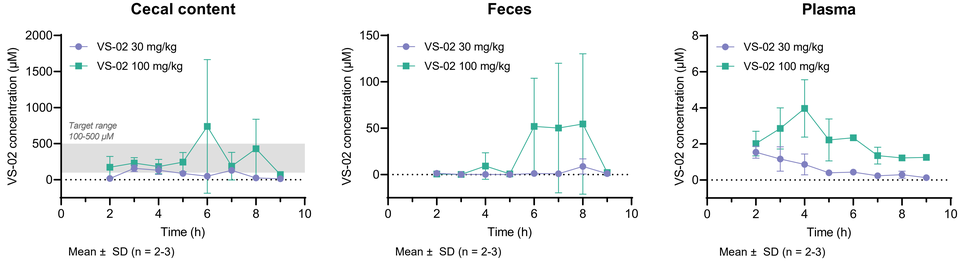

Figure 2: VS-02 PK profiles in cecal content, feces and plasma of healthy rats

→ **Effective local concentration (> 100 µM) in the cecum was achieved with 100 mg/kg VS-02 dose**

→ **VS-02 demonstrates high local exposure in cecal content, with plasma and feces levels representing less than 1% and ca. 2–4% of cecal content exposure, respectively, at both doses**

→ **C_{max} and AUC increased proportionally in all matrices (cecal content, feces, plasma) from 30 to 100 mg/kg dose**

Table 1: PK parameters following oral administration of 30 or 100 mg/kg VS-02

Dose (mg/kg)	Matrix	T_{max} (h)	C_{max} (µM)	C_{max}/Dose (µM*kg/mg)	$AUC_{0\text{-}9h}$ (µM/h)	$AUC_{0\text{-}9h}$/Dose (µM*kg/mg*h)	$AUC_{0\text{-}9h}$ (plasma or feces) / $AUC_{0\text{-}9h}$ (cecal content) (%)
30	Cecal content	3	158.3	5.28	597.2	19.9	-
	Feces	8	9.1	0.30	12.6	0.4	2.1
	Plasma	2	1.5	0.05	5.7	0.2	0.95
100	Cecal content	6	738.3	7.38	2314.5	23.1	-
	Feces	6	32.3	0.32	95.4	1.0	4.1
	Plasma	4	4	0.04	17.5	0.2	0.8

CONCLUSION

- **VS-02 demonstrated efficacy in a complex *ex vivo* bacterial system, showing greater potency compared to HAs previously investigated in clinical trials**
- **Preclinical formulation of VS-02 enabled targeted delivery of the inhibitor in the cecum and minimized systemic exposure supporting its further assessment in *in vivo* efficacy studies in animal models of HE**
- **Overall, these results support continued development of VS-02 as a potential treatment of HE in patients with cirrhosis**

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