AXA1665 Shows Dose-Dependent Alterations in Metabolic Profile, Including Reduction of Non-dosed Aromatic Amino Acids, That Differentiate it From Protein Supplement in Healthy Subjects

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Poster

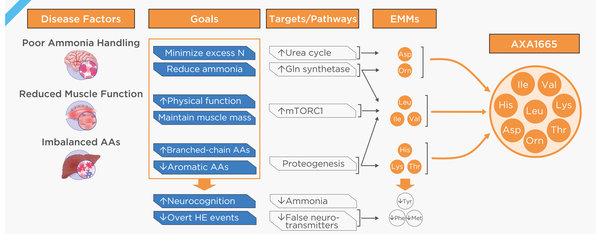
Virtual Café

Introduction

- Dysregulated nitrogen metabolism in cirrhosis, i.e., elevated plasma ammonia and amino acid imbalance (decreased branched chain amino acids [BCAAs] and elevated aromatic amino acids [ArAAs]), is associated with complications of sarcopenia and cognitive dysfunction[1-3]
- Impaired hepatic detoxification of ammonia leads to accelerated catabolism of BCAAs in skeletal muscle. This cascade contributes to the development of sarcopenia and to hepatic encephalopathy (HE) in patients with cirrhosis
- High-protein intake or protein supplementation is recommended as part of standard-of-care management of cirrhosis to counter muscle wasting, although concern remains about potential ammoniagenesis and ArAA elevation, which may exacerbate the risk of overt HE and associated outcomes
  - Dietary BCAA supplementation stimulates protein synthesis in skeletal muscle in cirrhosis and is effective in reducing episodes of HE,[4] but has the potential to enhance ammonia production in the intestine and kidney[5,6]
  - Dietary supplementation with the urea-cycle amino acids L-ornithine and L-aspartate (LOLA) lowers plasma ammonia and may also be of benefit in preventing HE[7]
- An alternative approach, using a unique stoichiometric ratio of specific amino acids to target multiple biologies, may be superior to protein supplementation in rebalancing cellular homeostasis in the liver and muscle
- AXA1665 is an investigational fixed-ratio composition of BCAAs (leucine, isoleucine, and valine), other proteogenic essential amino acids (histidine, lysine, and threonine), and urea-cycle amino acids (LOLA), which has shown clinical potential in mitigating the core metabolic derangements associated with cirrhosis[8,9] (Figure 1)

FIGURE 1

AXA1665: Hypothesized Mechanism of Action



AA, amino acid; Asp, aspartate; EMM, endogenous metabolic modulator; Gln, glutamine; HE, hepatic encephalopathy; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; mTORC1, mammalian target of rapamycin complex 1; N, nitrogen; Orn, ornithine; Phe, phenylalanine; Thr, threonine; Tyr, tyrosine; Val, valine.

Objective

- To support further development of AXA1665, this study was undertaken to characterize the plasma pharmacokinetics of the constituent amino acids within AXA1665 and their relative bioavailability compared with that of a commercially sourced protein supplement

Methods

Study Design

- In this open-label, single-dose, 4-way crossover study with ≥3-day washout, healthy subjects were randomized to 4 treatment sequences and received 1 of 4 treatments in a fasted state in each period: AXA1665 4.9 g, 9.8 g, 19.6 g, or protein supplement (Pure Pro® 35, American Body Building Products; 35 g protein)
- Blood samples were collected at –2, –1, and 0 hours pre-dose and up to 6 hours post-dose for assay (liquid chromatography with tandem mass spectrometry) of plasma amino acid concentrations

Pharmacokinetic Analysis

- Plasma pharmacokinetics were determined for 8 AXA1665-dosed and 12 AXA1665–non-dosed amino acids by noncompartmental analysis of baseline-corrected data using Phoenix® WinNonlin® (version 8.0)
- In the case of AXA1665-dosed amino acids, negative concentrations caused by baseline correction (signifying decreased post-dose vs predose exposure) were set to 0
- Area under the curve (AUC) for AXA1665-dosed amino acids was calculated from time 0 to the last quantifiable concentration (AUC_{last}), using positive baseline-corrected values
- AUC for non-dosed amino acids was calculated from time 0 to the last observed concentration (AUC_{all}), using both positive and negative baseline-corrected values (thereby accounting for instances in which decreased exposure was noted)

Results

Study Population

- Study subjects were healthy male (n=10) and female (n=6) nonsmokers, aged 19 to 47 (median 29.5) years, with a mean body mass index of 24.5 kg/m²

Amino Acid Content of Study Products

- High-performance liquid chromatography-based analysis of the amino acid content of protein supplement revealed qualitative differences from AXA1665, notably the additional presence of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and the absence of ornithine (Table 1)

Table 1: Composition of AXA1665 and Measured Amino Acid Content of Protein Supplement*

	AXA1665 4.9 g	AXA1665 9.8 g	AXA1665 19.6 g	Protein Supplement
Leucine	0.89	1.78	3.56	3.26
Isoleucine	0.44	0.89	1.78	1.78
Valine	0.89	1.78	3.56	2.12
Histidine	0.33	0.67	1.33	0.84
Lysine†	0.33	0.67	1.33	2.71
Threonine	0.33	0.67	1.33	1.47
Ornithine aspartate	1.67	3.33	6.67	2.56‡
Phenylalanine	–	–	–	1.69
Tyrosine	–	–	–	1.83
Tryptophan	–	–	–	0.43
Methionine	–	–	–	0.93
Glycine	–	–	–	0.64
Other amino acids§	–	–	–	15.18
Total amino acids	**4.9**	**9.8**	**19.6**	**35.4**

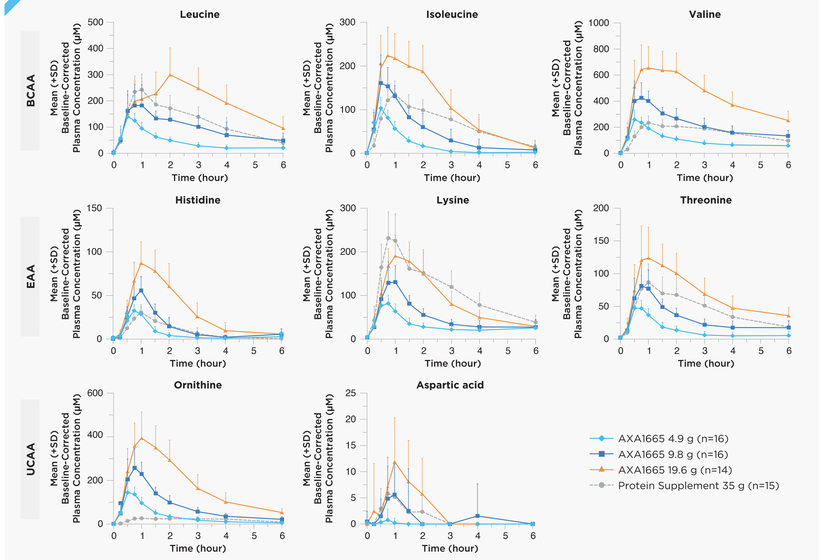
*Amounts for individual amino acids rounded to 2 decimal places; amounts for total amino acids rounded to 1 decimal place.
†Administered as lysine acetate.
‡Aspartate 2.56 g, ornithine level below limit of detection.
§Alanine, arginine, cystine, glutamic acid, proline, and serine combined.

Pharmacokinetics of AXA1665-Dosed Amino Acids

- Peak plasma concentrations of all AXA1665-dosed amino acids were reached within 2 hours after oral administration of AXA1665 or protein supplement (Figure 2)
- Plasma elimination half-life of AXA1665-dosed amino acids was short (median 0.8–1.9 hours)
- Oral absorption of aspartic acid was variable (coefficient of variation >40%), and only a limited number of positive baseline-corrected concentrations were noted

FIGURE 2

Baseline-Corrected Plasma Concentration Versus Time Profiles of Dosed Amino Acids After Single-Dose Administration of AXA1665 or Protein Supplement



Data are presented as arithmetic means (SD).
SD, standard deviation.

Pharmacokinetics of AXA1665–Non-dosed Amino Acids

- Plasma concentration vs time profiles for non-dosed amino acids showed dose-dependent decreases in phenylalanine, tyrosine, tryptophan, methionine, and glycine exposure, and dose-dependent increases in alanine, arginine, glutamine, and glutamic acid exposure after administration of AXA1665 4.9–19.6 g (Figure 3)
- In contrast, administration of protein supplement resulted in increased systemic exposure to the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Figure 3)

Relative Bioavailability Assessment of AXA1665 Versus Protein Supplement

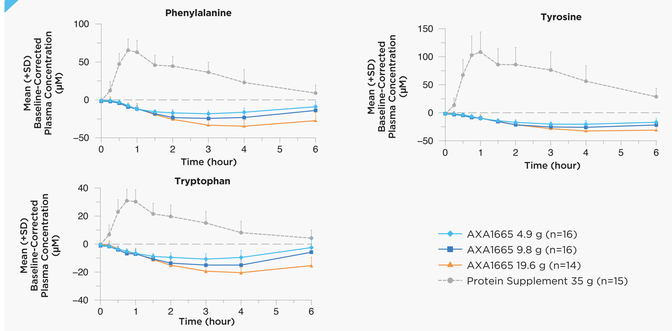
- Compared with protein supplement (35 g), AXA1665 19.6 g administration resulted in 1.5- to 9.5-fold higher systemic exposure (AUC) to all AXA1665-dosed amino acids except for aspartate and lysine, and lower exposure to all non-dosed amino acids except for glutamine and alanine (Figure 4)
- AXA1665 19.6 g delivered higher plasma concentrations of AXA1665-dosed amino acids compared with protein supplement (35 g), even after adjusting for differences in amino acid content between the 2 products

Safety and Tolerability

- Single-dose administration of AXA1665 4.9–19.6 g and protein supplement 35.4 g was well tolerated

FIGURE 3

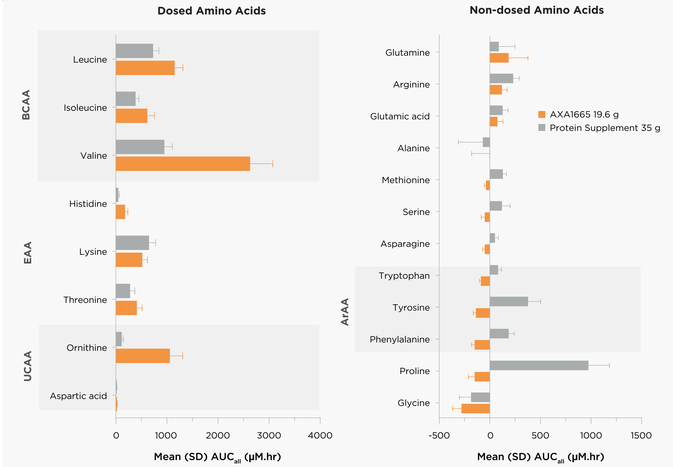
Baseline-Corrected Plasma Concentration Versus Time Profiles of Aromatic Amino Acids After Single-Dose Administration of AXA1665 or Protein Supplement (35 g protein)



Data are presented as arithmetic means (SD).
SD, standard deviation.

FIGURE 4

Increased Systemic Exposure to Dosed Amino Acids and Decreased Systemic Exposure to Non-dosed Amino Acids With AXA1665 19.6 g Compared With Protein Supplement



ArAA, aromatic amino acid; AUCall, area under the plasma concentration-time curve from time 0 to the time of the last observed concentration; BCAA, branched-chain amino acid; EAA, essential amino acid; SD, standard deviation; UCAA, urea cycle amino acid.

Discussion

- The pharmacokinetic findings indicate that AXA1665 administration results in increased plasma exposure to BCAAs, other proteogenic essential amino acids, and urea-cycle amino acids, and reduced plasma exposure to certain non-dosed ArAAs
- Reduction in plasma concentrations of endogenous ArAAs (phenylalanine, tyrosine) is expected to be of benefit in preventing overt HE recurrence by minimizing ammoniagenesis

Conclusions

- AXA1665 dose-dependently reduces plasma levels of non-dosed ArAAs, which are elevated in cirrhosis and implicated in the pathogenesis of overt HE, while increasing BCAAs, other essential amino acids, and urea cycle amino acids dose proportionally. In contrast, protein supplement increases systemic exposure to ArAAs
- AXA1665 has the potential to address underlying amino acid imbalances associated with overt HE in a targeted manner. AXA1665 is currently being evaluated in a phase 2 study in overt HE

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Disclosures

SV, NT, MK, SR: Employees of Axcella Therapeutics and may own stock options in the company. **JMcL, WC, MVC:** Employees of Axcella Therapeutics during the time the study was conducted.

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