Genetically engineered *E. coli* Nissle attenuates hyperammonemia in two experimental models of hepatic encephalopathy

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Introduction

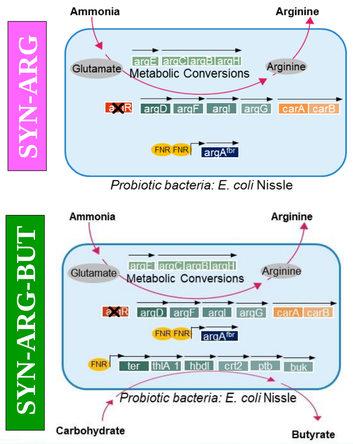
- Hepatic encephalopathy (HE) is a common and debilitating neuropsychiatric complication of chronic liver disease (CLD). HE, characterized by a constellation of symptoms, including cognitive, psychiatric and motor disturbances, can progress to coma and death. HE has a significant impact on the quality of life of patients and on their ability to function daily. As much as 80% of patients with cirrhosis suffer from some degree of HE.
- Hyperammonemia associated with CLD plays a major role in the pathogenesis of HE. Hence, lowering blood ammonia remains mainstay therapy for the treatment of HE.
- The gut is a major source of ammonia that contributes to systemic hyperammonemia, thus targeting the gut represents a potential approach to prevent the systemic absorption of ammonia.
- As a therapeutic strategy *E. coli* Nissle (EcN), a well characterized probiotic, was modified to convert ammonia to arginine (Arg) in the intestine by deleting a negative regulator of Arg biosynthesis and expressing a feedback resistant Arg biosynthetic enzyme. As an additional beneficial effect, EcN were engineered to synthesize butyrate, to reduce inflammation and maintain barrier integrity in the gut.
- Therefore, the rationale is that engineered EcN will continuously consume ammonia in the gut preventing its absorption into the blood.

Aim

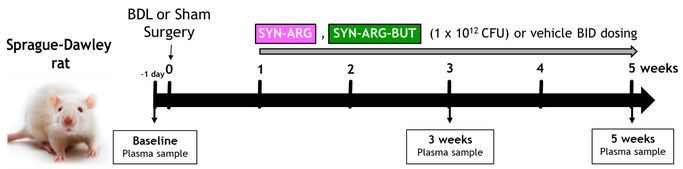
Explore the effects of engineered EcN on plasma ammonia levels in two experimental models of chronic liver disease and hyperammonemia: bile duct ligation and thioacetamide-induced liver injury

Methods

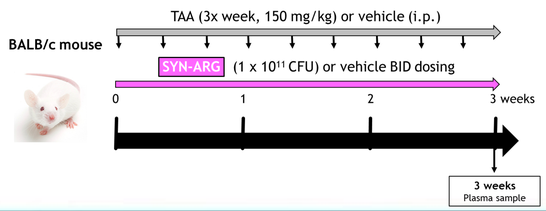
- Engineering EcN, therapeutic strategy to metabolize ammonia and produce butyrate:



- Bile duct ligation (BDL) was performed in rats in order to induce chronic liver disease and HE:

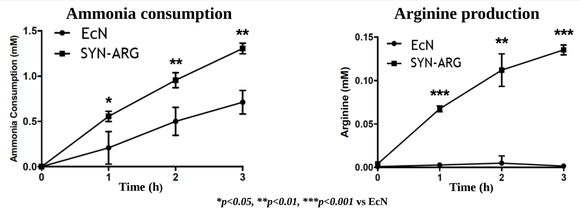


- Thioacetamide (TAA), an hepatotoxic drug, was used to induce liver fibrosis and hyperammonemia in mice:



Results

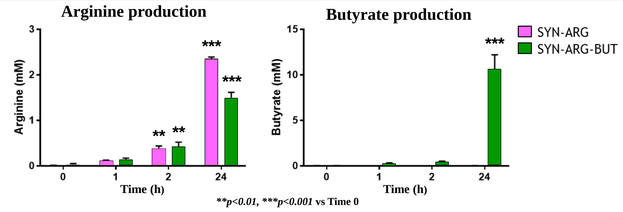
In vitro experiments: Engineered EcN SYN-ARG enhances the consumption of ammonia and produces arginine (Arg)



**p<0.05, **p<0.01, ***p<0.001 vs EcN*

An increase in ammonia consumption and arginine production was found in SYN-ARG compared to control EcN following exposure to ammonia (5mM).

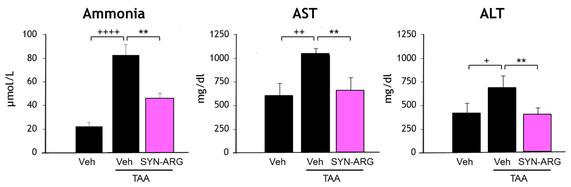
In vitro experiments: Arginine and butyrate production capabilities for SYN-ARG and SYN-ARG-BUT



***p<0.01, ***p<0.001 vs Time 0*

Both SYN-ARG and SYN-ARG-BUT generated arginine over time, while only SYN-ARG-BUT produced butyrate when exposed to ammonia (~18.7 mM).

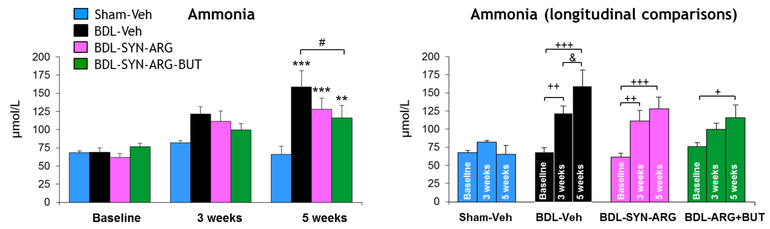
SYN-ARG prevents hyperammonemia and reduces liver injury in TAA mice



*+p<0.05, ++p<0.01, ++++p<0.0001 vs vehicle; **p<0.01 vs TAA-SYN-ARG*

Treatment with SYN-ARG significantly attenuated hyperammonemia as well as liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT).

SYN-ARG and SYN-ARG-BUT attenuates hyperammonemia in BDL rats

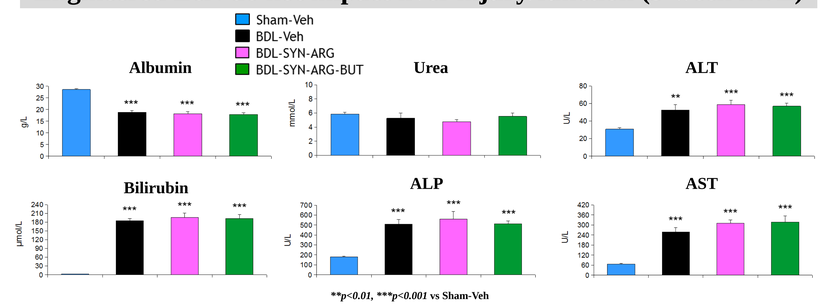


***p<0.01, ***p<0.001 vs Sham-Veh; #p<0.05 vs BDL-Veh*

+p<0.05, ++p<0.01, +++p<0.001 vs Baseline; &p<0.05 vs 3 weeks

At baseline and 3 weeks, no significant difference in blood ammonia was found between treated and non-treated groups. At 5 weeks, SYN-ARG-BUT significantly attenuated hyperammonemia with a trend observed in SYN-ARG vs BDL-Veh. An increase in blood ammonia was found in BDL-Veh at 3 + 5 weeks vs baseline with further increment at 5 weeks. Blood ammonia increased in SYN-ARG at 3 + 5 weeks vs baseline but no further increment at 5 weeks. Hyperammonemia was attenuated by SYN-ARG-BUT at 3 weeks but not at 5 weeks vs baseline.

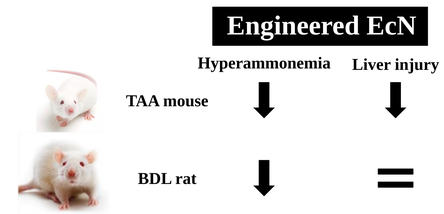
Engineered EcN do not impact liver injury/function (5-week BDL)



***p<0.01, ***p<0.001 vs Sham-Veh*

Albumin, bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) were increased in BDL groups with no effect from SYN-ARG or SYN-ARG-BUT.

Summary



Engineered EcN attenuates hyperammonemia in TAA and BDL models. Interestingly, liver injury is reduced in TAA mice but not in BDL rats. Therefore, results on TAA suggest that liver protection might contribute to the lowering ammonia effects of engineered EcN, while the effects on BDL rats suggest that the inhibition of ammonia absorption in the gut could be responsible.

Conclusion

- EcN, engineered to consume ammonia in the gut, is an effective approach to lower or prevent hyperammonemia in models of cirrhosis and hyperammonemia
- Data from BDL model suggest that the consumption of ammonia in the gut may explain the attenuation of hyperammonemia, while results from TAA model suggest an additional improvement in liver function
- The therapeutic potential of these engineered EcN strains should be further evaluated in patients with liver disease and HE

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