Tu2054

SYN-004, a Clinical Stage Oral Beta-Lactamase Therapy, Protects the Intestinal Microflora from Antibiotic-Mediated Damage in Humanized Pigs

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Abstract

Antibiotics (abx) that are excreted into the intestine, such as ceftriaxone (CRO), can damage the microflora and lead to serious illnesses such as *Clostridium difficile* infection. SYN-004 is a clinical stage oral beta-lactamase therapy for use with IV abx to preserve the microbiome by degrading residual abx within the intestine. Phase 1 clinical studies demonstrated safety and tolerability at all dose levels. Phase 2 was initiated in Q1, 2015 to assess intestinal CRO degradation in ileostomy subjects.

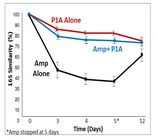
SYN-004 was engineered from the *B. licheniformis* PenP enzyme to broaden its abx hydrolysis spectrum. SYN-004 efficiently inactivates penicillins as well as a broad range of cephalosporins, including CRO, cefazolin, cefuroxime, and cefoperazone. SYN-004 was manufactured in *E. coli* and formulated into enteric-coated pellets. *In vitro*, the pellets remained intact at low pH (0.1 N HCl) and complete dissolution occurred at pH >5.5. In human chyme, SYN-004 enzyme activity was maintained for at least 6 hrs, demonstrating enzyme stability in human intestinal contents. Efficacy studies performed with CRO-treated (IV, 30 mg/kg) jejunal-fistulated dogs revealed high intestinal CRO levels (mean C_{max} of 1500 ug/g chyme at 90 min) that were completely eliminated in the presence of SYN-004 (≤5ug/g chyme). The ability of SYN-004 to protect the intestinal microbiome from CRO-induced damage was evaluated in a preliminary study in humanized pigs. The GI tract of 5 day old gnotobiotic pigs was populated with human adult fecal microflora. Two days later, animals received CRO (IP, 50 mg/kg) for 4 days. SYN-004 was delivered orally 4 times a day for 7 days beginning the day before CRO administration. Microbiome changes were monitored by high-throughput sequencing of the 16S rRNA gene V1V2 region using fecal DNA. The levels of a specific bacterial population expected to be sensitive to CRO, ampicillin-resistant aerobes including those of the phylum Proteobacteria, was assessed by plating equal amounts of feces on LB+amp plates. The figure displays the phylum-level taxonomic classifications and the quantification of the LB+amp bacterial growth. The Control (No Abx) and CRO+SYN-004 cohorts showed good representation by Bacteroidetes, Proteobacteria and Firmicutes, while the CRO alone cohort displayed dysbiosis, with Bacteroidetes as the greatly predominant phylum. The LB+amp data corroborate these findings, as the CRO+SYN-004 cohort displayed similar, high bacteria levels as the Control, while the CRO alone cohort displayed at least 2 log lower levels, suggesting a reduction in the Proteobacteria population.

These data demonstrate that SYN-004 has the potential to protect the human microbiome and to become the first prophylactic therapy designed to prevent abx-mediated microbiome damage, including *Clostridium difficile* infection, in patients receiving beta-lactam abx.

Background

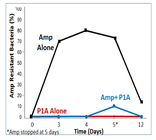
The β-lactam antibiotics excreted via the bile duct into the intestine can disrupt the intestinal microflora. In clinical trials, the β-lactamase, P1A, given orally with IV penicillins preserved the diversity of the intestinal microbiome, reduced the selection for antibiotic-resistant coliforms, efficiently degraded piperacillin/tazobactam in the intestine, and did not alter plasma antibiotic levels. However, P1A has limited utility as it does not efficiently degrade cephalosporins, use of which is a major risk factor for C. *difficile* infection.

Similarity Index



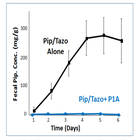
16S similarity index significantly different between Amp Alone and Amp+P1A

Amp-Resistant Bact.



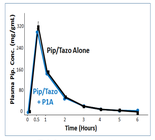
Rates of amp resistance remained below 10% for Amp+ P1A

GI Tract



P1A degraded Pip/Tazo in GI tract

Systemic



P1A did not alter antibiotic plasma levels

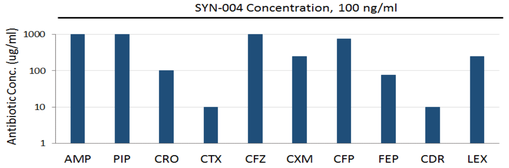
SYN-004, engineered from P1A with a one aa change (D276N), displays a broad antibiotic degradation profile, efficiently degrades ceftriaxone in the GI tract of dogs, and protects the microbiome in neonatal humanized pigs. Clinical evaluation of SYN-004 was initiated in 2014 and demonstrated safety and tolerability of SYN-004 at all dose levels. Additional clinical studies are ongoing.

Results

SYN-004 Antibiotic Degradation Profile

SYN-004 was evaluated for antibiotic inactivation with a microtiter plate activity assay using E. *coli* growth as the read-out for antibiotic degradation.

Antibiotic Hydrolysis



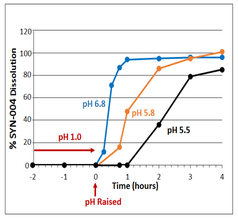
AMP:ampicillin; PIP:pipercillin; CRO:ceftriazone; CTX:cefotaxime; CFZ:cefozolin; CXM:cefuroxime; CFP:cefoperazone; FEP:cefepime; CDR: cefdinir; LEX: cephalexin

SYN-004 displayed activity against penicillins and cephalosporins.

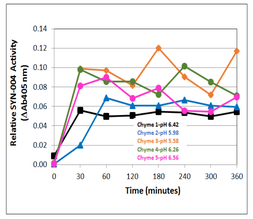
SYN-004 Dissolution and Stability in Human Chyme

SYN-004 was manufactured in E. *coli,* and formulated into enteric-coated pellets that were used to fill capsules. SYN-004 pellets were held in a 0.1 N HCL solution for 2 hrs followed by incubation in buffers at pHs 6.8, 5.8, or 5.5 from 0.25 to 4 hrs. Human chyme from five different donors was characterized based on pH, liquid content, and protease activities. SYN-004 pellets were incubated in each chyme from 30 to 360 min. SYN-004 activity was assessed using a CENTA chromogenic assay.

pH Dissolution Profile



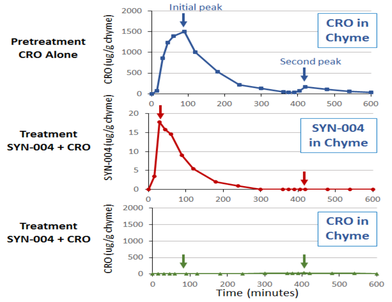
Stability in Human Chyme



SYN-004 pellets were protected at low pH (conditions expected in the stomach) while dissolution occurred at pHs > 5.5, with pHs 6.8 and 5.8 showing more rapid dissolution than pH 5.5. In human chyme, SYN-004 pellets showed rapid dissolution, within 30-60 min. High-level SYN-004 activity was observed for at least 6 hours, demonstrating SYN-004 enzyme stability in human chyme.

SYN-004 Degraded Ceftriaxone in the GI Tract of Dogs

SYN-004 was tested in the intestinal tract of jejunal-fistulated dogs (n=6) following oral delivery of SYN-004 enteric-coated pellets (0.44 mg/kg) and IV ceftriaxone (30 mg/kg).

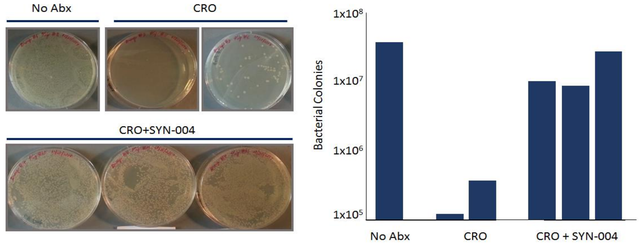


The dog studies revealed that ceftriaxone (CRO) was excreted at high levels into the intestine following IV delivery and a second CRO peak was observed after an additional feeding (at 6 hrs). SYN-004 delivered orally 10 min prior to IV CRO, eliminated the initial peak of CRO in the intestine of 4/6 dogs (graph displays data from the 4 dogs), and the second peak of CRO in 6/6 dogs. These data demonstrate that SYN-004 was present, remained functional, and hydrolyzed the CRO in the intestines of all treated dogs.

SYN-004 Protected the Intestinal Microflora of Humanized Gnotobiotic Pigs

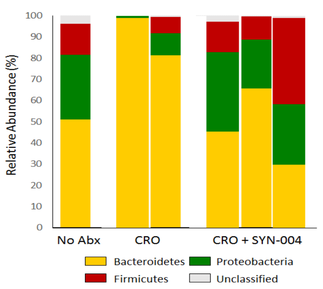
The GI tract of 5 day old gnotobiotic pigs was populated with human adult fecal microflora. Animals received ceftriaxone (CRO) once a day (IP, 50 mg/kg) 2 days later for 4 days. SYN-004 was delivered orally 4 times a day for 7 days beginning the day before CRO delivery. Changes to the microbiome were monitored in two ways. First, the levels of a specific bacterial population expected to be sensitive to CRO, ampicillin-resistant aerobes, were assessed by plating equal amounts of feces on LB+amp plates. Second, fecal DNA from samples collected 3 days after antibiotics were stopped was subjected to 16S rRNA V6 region sequencing.

Assessment of Ampicillin-Resistant Aerobes



The No-Antibiotic Control (No Abx) and the CRO+SYN-004 cohorts displayed similar, high bacteria levels. In contrast, the CRO alone cohort displayed at least 2 log lower bacterial levels. As bacteria from the phylum Proteobacteria are most likely the bacteria sensitive to CRO, these data suggest that CRO depletes the Proteobacteria population while SYN-004 protects this bacterial population in the gut from the effects of CRO.

Phylum-Level Taxonomic Classification of GI Microflora



16S rRNA V6 region sequence analysis of fecal DNA revealed that the Control (No Abx) and CRO+SYN-004 cohorts showed good representation by Bacteroidetes, Proteobacteria, and Firmicutes, while the CRO alone cohort displayed dysbiosis with Bacteroidetes as the most predominant phylum.

SYN-004 protected the GI microflora of humanized pigs from dysbiosis caused by CRO antibiotic use.

Conclusions

- SYN-004 efficiently degrades penicillins and a panel of cephalosporins, including ceftriaxone
- Enteric-coated SYN-004 pellets are inert at low pH and rapidly dissolve at pHs >5.5
- Enteric-coated SYN-004 pellets rapidly dissolve in human chyme with stable activity for >6 hours
- In dogs, oral delivery of SYN-004 pellets resulted in efficient degradation of intestinal ceftriaxone
- In humanized neonatal pigs, SYN-004 protected the intestinal microflora from dysbiosis caused by ceftriaxone
- These data demonstrate that SYN-004 has the potential to protect the human microbiome and to become the first prophylactic therapy designed to prevent antibiotic-mediated microbiome damage, including *C. difficile* infection, in patients receiving beta-lactam antibiotics

Disclosures

S. Connelly, J.A. Bristol, S. Hubert, J. Sliman, and M. Kaleko are employees of Synthetic Biologics, Inc. Synthetic Biologics, Inc. sponsored the humanized neonatal pig study performed at Tufts.