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 (β-lactams) that are excreted into the intestine, such as ceftriaxone (CRO), can damage the microbiome and lead to various illnesses such as Clostridium difficile infection. SYN-004 is a clinical stage oral beta-lactamase inhibitor that, in our preclinical studies, prevented the microbiome from degrading antibiotic residues within the intestine. Phase 2a was initiated in Q1, 2015 to assess clinical β-lactam damage in elderly patients. SYN-004 was engineered from Pseudomonas aeruginosa via reverse genetic cloning into the β-lactamase gene to confer antibiotic resistance to a series of β-lactams, allowing therapeutic use in the presence of antibiotic residues in the human gut. The parent molecule, ceftriaxone (CRO), was used across all studies to evaluate the efficacy of SYN-004 in preventing antibiotic-mediated damage. SYN-004 exposed higher intestinal β-lactamase activity in SYN-004 animals, and was completely eliminated from the gut at 48 h. CRO-induced damage was mitigated in a preliminary study in humanized pigs. The GI tract of 2-day old piglets was populated with human adult fecal mixed inoculum. Test meal (enalapril coated CRO) was administered, and antibiotic levels were monitored by high-throughput sequencing of 16S rRNA gene amplicons in fecal pellets collected at 24 h and 3 days post treatment. Intestinal β-lactamase activity was determined using 200 β-lactamase-positive enteric anaerobes among those of the phylum Proteobacteria, was assessed by pooling equal quantities of fecal pellets from 4 infant pigs. After administration, β-lactamase activity was assessed. The study showed that SYN-004 was effective in preventing antibiotic-mediated damage in both human and piglets. These data demonstrate that SYN-004 has the potential to protect the human microbiota and to become the first prophylactic therapy designed to prevent antibiotic-mediated microbiome damage. SYN-004 is currently in a phase 1 clinical trial to assess safety in patients receiving beta-lactam antibiotics.

Background

The β-lactam antibiotics excreted via the bile duct into the intestine can disrupt the intestinal microbiota. In clinical trials, the lactamase, P. aeruginosa, with only 4% pyrrolidines preserved the diversity of the intestinal microbiome, reduced the selection for antibiotic-resistant coliforms, efficiently degraded cephalosporin to lactam in the intestine, and did not alter plasma antibiotic levels. However, P. aeruginosa has limited utility as it does not efficiently degrade cephalosporins, use of which is a major risk factor for C. difficile infection.

SYN-004, engineered from P. aeruginosa with one aa change (D276N), displays a broad antibiotic degradation profile, efficiently degrades cephalosporin in the GI tract of neonates, protects the microbiota 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.

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

Results

SYN-004 Degraded Ceftriaxone in the GI Tract of Dogs

SYN-004 was tested in the intestinal tract of julian-flasted dogs (n=6) following oral delivery of SYN-004 enteric-coated pellets (0.44 mg/kg) and 4 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 h); SYN-004 delivered orally 10 min prior to IV CRO, eliminated the initial peak of CRO in the intestine of 4 dogs (graph displays data from the 4 dogs), and the second peak of CRO in 6 dogs. These data demonstrate that SYN-004 was present, remained functional, and hydrolyzed the CRO in the intestines of all treated dogs.

Phylum-Level Taxonomic Classification of GI Microbiota

16S RNA V6 region sequence analysis of fecal DNA revealed that the CRO (No Abx) and CRO+SYN-004 cohorts showed great representation by Bacteroidetes, Proteobacteria, and Firmicutes; the CRO alone cohort displayed a greater presence with Bacteroidetes as the most predominant phylum.

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 > 6.5.
- 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 microbiota 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.