Oral Beta-Lactamase Therapies Prevent Microbiome Damage and Attenuate Antibiotic Resistance from IV and Oral Antibiotics in Large Animal Models of Antibiotic-Mediated Gut Dysbiosis

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ABSTRACT

Background: Antibiotics can damage the gut microbiome leading to overgrowth of pathogens and provide selective pressure for emergence of antibiotic resistance. SYN-004 (ribaxamase) is a clinical-stage beta-lactamase formulated for oral delivery intended to degrade certain beta-lactam antibiotics in the GI tract to preserve the gut microbiome. Ribaxamase was evaluated in a phase 2b clinical study that met its primary endpoint of significantly reducing *C. difficile* infection in patients treated with IV ceftriaxone and demonstrated protection of the gut microbiome with reduced emergence of antibiotic resistance. Ribaxamase is intended for use with IV penicillins and cephalosporins, but does not degrade carbapenems. Beta-lactamase-mediated microbiome protection was expanded to include oral and carbapenem antibiotics.

Methods: For use with oral beta-lactams, a ribaxamase formulation, SYN-007, was engineered for release in the lower small intestine, distal to the site of antibiotic absorption. For use with IV carbapenems, SYN-006, a novel metallo-beta-lactamase, was formulated for oral delivery. SYN-007 (10 mg, PO, TID) was evaluated in dogs treated with oral amoxicillin (40 mg/kg, PO, TID) for 5 days. SYN-006 (50 mg, PO, QID) was evaluated in pigs treated with ertapenem (30 mg/kg, IV, SID) for 4 days. Serum antibiotic levels were measured and fecal DNA whole genome shotgun sequence analyses were performed.

Results: In dogs and pigs, systemic antibiotic levels were not significantly different +/- SYN-007 or SYN-006. Fecal DNA metagenomics analyses demonstrated that oral amoxicillin and IV ertapenem resulted in significant changes to the gut microbiome. SYN-007 and SYN-006 attenuated microbiome damage and reduced emergence of antibiotic resistance.

Conclusion: Ribaxamase, SYN-007, and SYN-006 have the potential to protect the commensal gut microbiota from antibiotic-mediated collateral damage and to mitigate emergence and spread of antibiotic resistance, thereby broadening the utility of this prophylactic approach to include all classes of beta-lactam antibiotics, delivered both systemically and orally. Antibiotic inactivation represents a new paradigm for preservation of the gut microbiome and reduction of antibiotic resistance.

BACKGROUND

IV beta-lactam antibiotics, including cephalosporins, are excreted via the bile into the intestine where they can disrupt the intestinal microbiota and potentially lead to the outgrowth of pathogens like *Clostridium difficile*. SYN-004 (ribaxamase), a clinical stage, oral beta-lactamase enzyme therapy for use with IV beta-lactam antibiotics designed to preserve the gut microbiota by degrading residual antibiotics in the GI tract. A phase 2b study met its primary endpoint of significantly reducing *C. difficile* infection (CDI) in patients treated with ceftriaxone and ribaxamase.

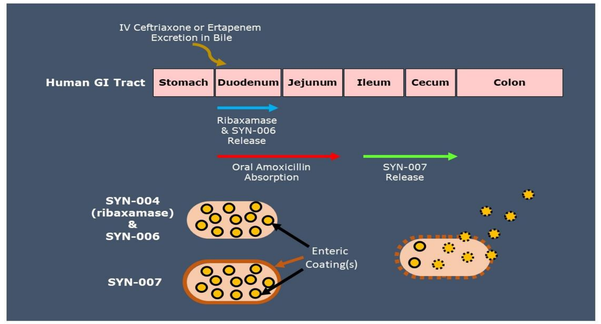
The ribaxamase clinical formulation consists of enteric-coated enzyme pellets engineered to protect the enzyme from stomach acid with enzyme release occuring at pH > 5.5, in the upper small intestine. As expected, use of the clinical formulation with oral amoxicillin in pigs prevented systemic absorption of the antibotic presumably due to degradation of the antibiotic prior to its absorption. SYN-007 was developed as a delayed release formulation of ribaxamase, intended to release enzyme in the GI tract at a site distal oral antibiotic absorption.

SYN-006, a novel metallo-beta-lactamase, derived from *Bacillus cereus*, able to degrade all classes of beta-lactam antibiotics including carbapenems, was manufactured and formulated into enteric-coated enzyme pellets for oral delivery. As carbapenem antibiotics are delivered parenterally, the carbapenemase was formulated for release in the upper small intestine, similar to the clinical ribaxamase clinical formulation.

RESULTS

Enteric Coated Beta-Lactamase Formulations

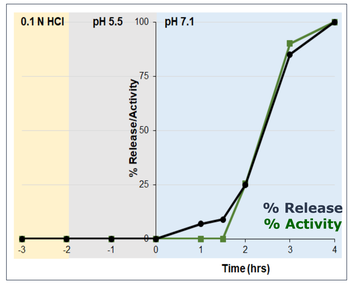
SYN-007 is a distal-release formulation of the beta-lactamase, ribaxamase, engineered for lower small intestine dissolution distal to the site of oral antibiotic absorption and contains enteric-coated ribaxamase pellets within enteric-coated capsules engineered for release at pH >7.0. SYN-006 consists of enteric coated metallo-beta-lactamase pellets intended to protect the ezyme from low pH and to dissolve at pH > 5.5 for release in the upper small intestine.



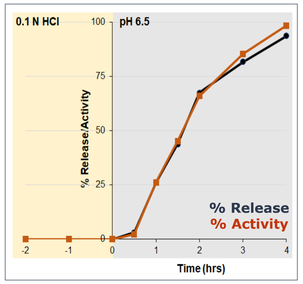
Dissolution Under Differing pH Conditions

Dissolution of SYN-007 and SYN-006 was tested in vitro. SYN-007, designed to be released in the distal small intestine, is composed of enteric-coated enzyme pellets within enteric-coated capsules. SYN-006, designed to be released in the proximal small intestine is enteric-coated enzyme pellets in uncoated capsules. For SYN-007, capsules were held in 0.1N HCl (pH 1.1) for 1 hr to simulate conditions in the stomach, 2 hrs at pH 5.5, conditions of the upper small intestine, and 4 hrs at pH 7.1, conditions of the lower small intestine/colon. For SYN-006, capsules were held in 0.1 N HCl for 2 hrs, and pH 6.5 for 4 hrs.

SYN-007



SYN-006

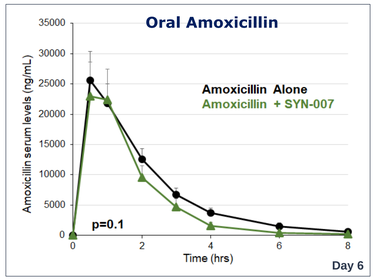


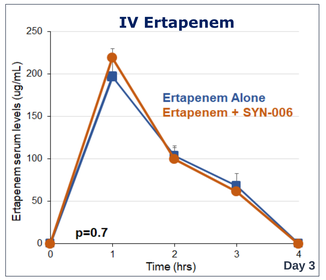
No enzyme release was detected in 0.1N HCl for both formulations. For SYN-007, no release occurred at pH 5.5, and release occurred slowly at pH 7.1 for the first 2 hrs, with complete dissolution by 4 hrs. For SYN-006, dissolution occurred uniformly at pH 6.5, with complete release by 4 hrs. For both SYN-007 and SYN-006, enzyme release paralleled enzyme activity, indicating that the formulated beta-lactamases retained full biological activity.

Beta-Lactamases Do Not Affect Systemic Antibiotic Levels

Dogs received 16 doses of oral amoxicillin +/- SYN-007 (TID). Blood was collected after the first and last dose. Pigs received 4 doses of IV ertapenem (SID) +/- SYN-006 (QID, starting the day before ertapenem administration for a total of 21 doses). Blood was collected on day 3, after 3 doses of ertapenem. Serum was analyzed for antibiotic levels.

Antibiotic Serum Pharmacokinetics



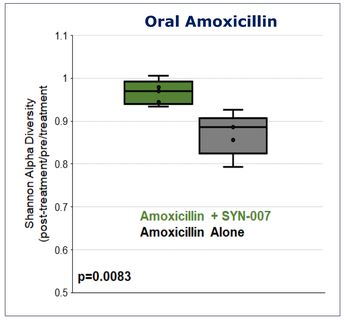


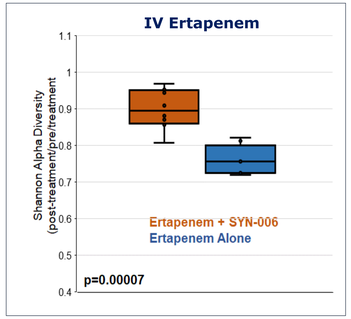
For SYN-007, amoxicillin serum levels were not significantly different in the presence or absence of SYN-007, indicating that SYN-007 did not interfere significantly with amoxicillin systemic absorption. For SYN-006, ertapenem serum levels were not significantly different in the presence or absence of SYN-006, indicating that SYN-006 did not interfere with ertapenem blood levels.

SYN-007 and SYN-006 Protect the Gut Microbiome

For microbiome analyses, fecal DNA, collected prior to and after the last antibiotic +/- SYN-007 or SYN-006 doses, was subjected to whole genome shotgun sequence analyses using CosmosID, Inc. metagenomics software.

Shannon Alpha Diversity





Both SYN-007 and SYN-006 protected the gut microbiome from collateral damage caused by oral amoxicillin and IV ertapenem, respectively.

Microbiome Shannon alpha diversity was significantly greater in the presence of SYN-007 (p=0.0083) and SYN-006 (p=0.00007) compared to antibiotic alone (Student T-test, two-tailed, unpaired, unequal variance).

CONCLUSIONS

- Ribaxamase is intended as an orally-delivered beta-lactamase to protect the gut microbiome from IV beta-lactam antibiotic-mediated dysbiosis
- A phase 2b clinical study met its primary endpoint of significantly reducing *C. difficile* disease in patients receiving IV ceftriaxone + ribaxamase
- To expand protection to include oral beta-lactams and carbapenems new ribaxamase formulations and/or a novel beta-lactamase were developed

SYN-007 is a distal-release formulation of ribaxamase designed for use with oral beta-lactam antibiotics

In dogs, SYN-007:

- Did not interfere with oral amoxicillin absorption
- Protected gut microbiota from amoxicillin damage
- Reduced emergence of antibiotic resistance genes

SYN-006 is an enteric-coated formulation of a novel carbapenemase

In pigs, SYN-006:

- Did not interfere with IV ertapenem systemic levels
- Protected gut microbiota from ertapenem damage
- Reduced emergence of antibiotic resistance genes

SYN-007 and SYN-006 have the potential to expand microbiome protection to oral, as well as IV beta-lactam antibiotics, and all classes of beta-lactams including penicillins, cephalosporins, and carbapenems

DISCLOSURES

SC, CFF, and MK are employees of Synthetic Biologics, Inc. RRC is the founder of CosmosID, Inc., a fee-for-service provider engaged by Synthetic Biologics, Inc. BF and NAH are employees of CosmosID, Inc. This work was supported by Synthetic Biologics, Inc.