Orally Delivered Beta-Lactamase Prevents Gut Microbiome Dysbiosis Caused by IV and Oral Antibiotics and Mitigates Propagation of Antibiotic Resistance in Porcine and Canine Models

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ABSTRACT

Disruption of the intestinal microbiome is an unintended consequence of antibiotic use that can lead to overgrowth of pathogenic organisms and spread of antibiotic resistance. SYN-004 (ribaxamase) is a clinical-stage, oral beta-lactamase for use with selected IV beta-lactam antibiotics intended to preserve the gut microbiota by inactivating residual antibiotics in the intestine. A Phase 2b study assessing ribaxamase efficacy in patients receiving IV ceftriaxone met its primary endpoint of significantly reducing *C. difficile* infection and diminishing emergence of vancomycin-resistant enterococci. In a porcine model of antibiotic-mediated dysbiosis, ribaxamase protected the gut microbiome from IV ceftriaxone and reduced emergence of antibiotic resistance. In this study, delayed release formulations of ribaxamase, named SYN-007, were tested in dogs treated with oral amoxicillin.

SYN-007 was engineered for release in the lower small intestine distal to the site of oral amoxicillin absorption. Multiple formulations of SYN-007, composed of enteric-coated enzyme pellets within enteric-coated capsules, were evaluated for pH-mediated dissolution *in vitro*, and three were chosen for *in vivo* testing. Dogs received amoxicillin (40 mg/kg, PO, TID) +/- SYN-007 (10 mg, PO, TID) for 5 days. Amoxicillin serum levels were measured by LC/MS/MS after the first and last doses. DNA, isolated from feces collected before and after antibiotic treatment, was subjected to whole genome shotgun sequence analyses using CosmosID, Inc. metagenomics software. Serum amoxicillin levels were not significantly different +/- SYN-007 after the first dose for all SYN-007 formulations, while only one SYN-007 formulation resulted in similar systemic antibiotic concentrations after the last dose. Gut microbiomes of animals that received amoxicillin alone revealed loss of some bacterial species and overgrowth of others, and emergence of a broad spectrum of antibiotic resistance genes. In contrast, in animals that received amoxicillin + SYN-007, changes to the gut microbiota were diminished and antibiotic resistance gene frequencies were reduced.

Oral amoxicillin caused significant changes to the gut microbiome and triggered emergence and propagation of antibiotic resistance genes in dogs. SYN-007 diminished amoxicillin-mediated microbiome disruption and mitigated emergence and propagation of antibiotic resistance genes in dogs. One SYN-007 formulation did not affect amoxicillin serum levels on day 5 indicating that SYN-007 was not released in the upper small intestine and did not degrade amoxicillin prior to its systemic absorption. Antibiotic inactivation represents a new treatment paradigm for preservation of the gut microbiome and reduction of antibiotic resistance. SYN-007 has the potential to expand beta-lactamase-mediated microbiome protection to oral as well as IV beta-lactam antibiotics.

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 outgrowth of pathogens like *Clostridium difficile*. SYN-004 (ribaxamase) is a clinical stage, oral beta-lactamase enzyme therapy for use with IV beta-lactam antibiotics designed to preserve the gut microbiome 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.

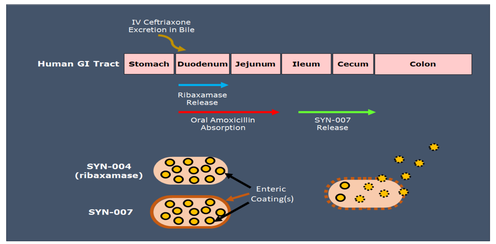
The ribaxamase clinical formulation consists of enteric-coated enzyme pellets engineered to protect the enzyme from stomach acid with enzyme release occuring at pH >5.5 in the upper small intestine. As expected, use of the clinical formulation with oral amoxicillin in pigs prevented systemic absorption of the antibotic, presumably due to degradation of the antibiotic prior to absorption.

Novel formulations of ribaxamase, named SYN-007, intended to release enzyme in the GI tract at a site distal to oral antibiotic absorption, were developed and evaluated *in vitro* and in dogs. One SYN-007 formulation did not interfere with amoxicillin absorption and protected the gut microbiome from antibiotic collateral damage, including maintaining microbiota diversity and reducing propagation of antibiotic resistance in dogs.

RESULTS

Distal Release Ribaxamase Formulations

Distal-release formulations of the beta-lactamase, ribaxamase, were engineered for dissolution in the lower small intestine distal to the site of oral antibiotic absorption.

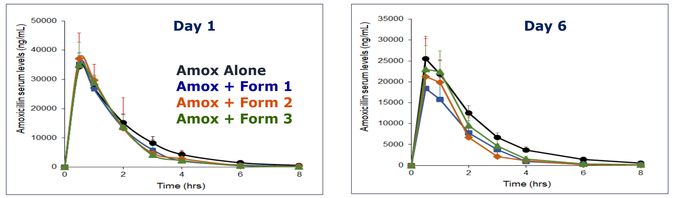


Three distal-release formulations of ribaxamase, SYN-007, were produced. Formulations 1 and 3 were composed of enteric-coated enzyme pellets within enteric-coated capsules. Formulation 2 contained enteric-coated pellets in uncoated capsules. Dissolution testing verified that enzyme was protected at low pH (conditions in the stomach), was not released after 2 hrs at pH 5.5, conditions of the upper small intestine, and was released at pH 7.1, conditions of the lower small intestine/colon. In contrast, SYN-004 is released at pH 5.5.

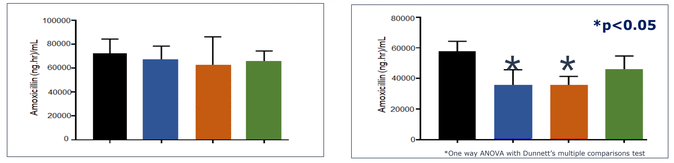
SYN-007 Formulation 3 Does Not Affect Amoxicillin Systemic Absorption

SYN-007 formulations were evaluated in dogs (n=5/cohort) that received oral amoxicillin (40 mg/kg, TID) +/- SYN-007 formulations 1, 2, or 3 (10 mg, PO, TID) for 5 days. Serum amoxicillin levels were measured after the first and last antibiotic doses to determine if SYN-007 interfered with amoxicillin systemic absorption.

Amoxicillin Serum Pharmacokinetics



Area Under the Curve

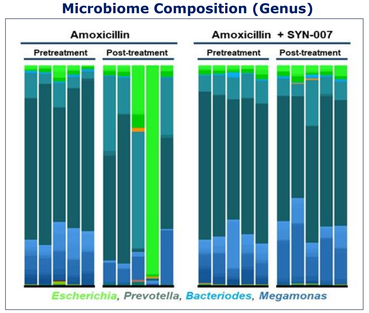


At Day 1, amoxicillin serum levels were not significantly different in the presence or absence of each formulation of SYN-007. In contrast, at Day 6, amoxicillin serum levels were significantly reduced with SYN-007 formulations 1 (p=0.0013) and 2 (p=0.0016), but were not significantly different with formulation 3 (p=0.0980), indicating that SYN-007 formulation 3 did not interfere significantly with amoxicillin systemic absorption.

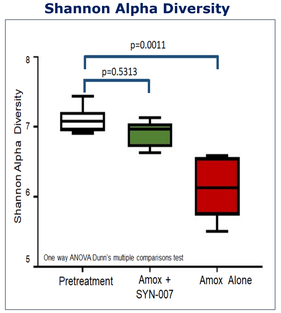
SYN-007 Protects the Gut Microbiome from Amoxicillin

For microbiome analyses, feces were collected prior to and after the last antibiotic +/- SYN-007 doses (16 doses of each total). Fecal DNA was subjected to whole genome shotgun sequence analyses using the CosmosID, Inc. metagenomics software.

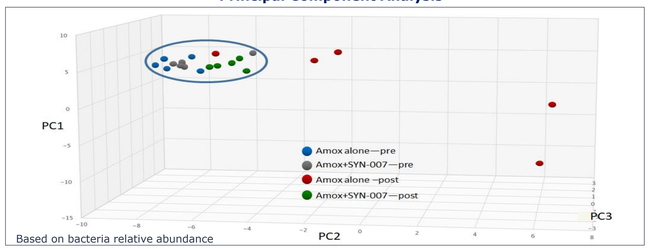
Microbiome Composition (Genus)



Shannon Alpha Diversity



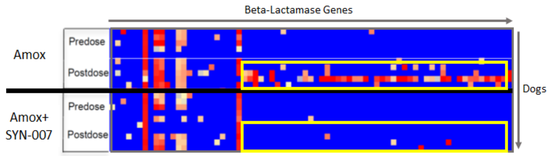
Principal Component Analysis



Microbiome composition was significantly changed after amoxicillin exposure. Microbiomes exposed to amoxicillin + SYN-007 were not significantly different from pretreatment, demonstrating that SYN-007 protected the microbiomes from amoxicillin damage.

SYN-007 Reduces Emergence of Beta-Lactamase Genes

Resistome heatmaps were generated based on the relative frequency of antibiotic resistance genes in each sample. This heatmap displays beta-lactamase gene frequency.

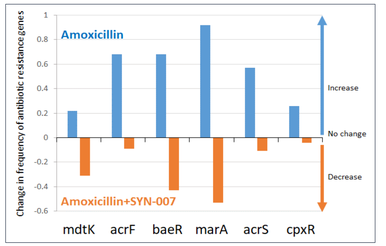


Emergence of beta-lactamase genes was reduced in the presence of SYN-007.

SYN-007 Reduces Emergence of Antibiotic Resistance Genes

In addition to beta-lactamase genes, other resistance genes displayed an increased frequency after antibiotic exposure. Change in relative frequency (mean) of the indicated antibiotic resistance genes is displayed.

Changes in AR Gene Frequency



Frequency of selected genes, encoding components of multidrug efflux transporters, increased after amoxicillin exposure and decreased in the presence of SYN-007. SYN-007 reduced the frequency of a broad spectrum of resistance genes, not only those conferring resistance to amoxicillin.

These data demonstrate that SYN-007 protected the gut microbiome from collateral damage caused by oral amoxicillin administration and reduced the emergence of a broad spectrum of antibiotic resistance genes in dogs.

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
- SYN-007 is a new, distal-release formulation of ribaxamase designed for use with oral beta-lactam antibiotics
- SYN-007 Form 3 did not interfere with oral amoxicillin absorption in dogs
- SYN-007 protected the gut microbiota from damage caused by amoxicillin in dogs
- SYN-007 reduced emergence of antibiotic resistance genes in dogs

SYN-007 has the potential to expand microbiome protection to include oral as well as IV beta-lactam antibiotics

DISCLOSURES

MK, CFF, and SC 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.