Gut Antibiotic Inactivation by Beta-Lactamases is Intended to Prevent Microbiome Damage and Attenuate Antibiotic Resistance in Large Animal Models

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ABSTRACT

Background: Exposure of the gut microbiome to antibiotics can harm the microbiota and lead to antibiotic resistance. SYN-004 (ribaxamase) is a clinical-stage, oral beta-lactamase enzyme intended to preserve the gut microbiome by inactivating certain IV beta-lactam antibiotics in the GI tract without affecting antibiotic infection control efficacy. The use of ribaxamase for microbiome protection from both IV and orally-delivered antibiotics was explored using two large animal models of antibiotic-mediated gut dysbiosis.

Methods: Ribaxamase was manufactured as enteric-coated pellets for pH-mediated release in the upper GI tract. New formulations, named SYN-007, were engineered to be released in the GI tract at a point distal to oral antibiotic absorption. Ribaxamase and SYN-007 were evaluated in a pig model of antibiotic-mediated microbiome disruption, and SYN-007 was evaluated in dogs. Pigs were treated with ceftriaxone (CRO; IV, 50 mg/kg, SID) or amoxicillin (AMX; PO, 20 mg/kg, BID) for 7 days +/- ribaxamase (PO, 75 mg, QID). Dogs received AMX (80 mg/kg, PO) +/- SYN-007 (10 mg, PO). Serum antibiotic levels were measured via HPLC or LC/MS/MS, and fecal DNA whole genome shotgun (WGS) sequence analyses were performed with CosmosID metagenomics software.

Results: In pigs, ribaxamase protected the gut microbiome from IV CRO and oral AMX and reduced propagation of antibiotic resistance genes. Ribaxamase had no effect on CRO serum levels. In contrast, AMX was not detected in the serum when delivered with ribaxamase indicating that AMX was degraded in the GI tract prior to its absorption. Delivery of delayed-release SYN-007 with oral AMX in dogs did not affect AMX absorption, as AMX serum pharmacokinetics (PK) were similar with and without SYN-007.

Conclusion: Ribaxamase protected the gut microflora in pigs from damage caused by IV CRO and reduced emergence of antibiotic resistance genes. SYN-007, a novel formulation designed to target enzyme release distal to the site of AMX absorption, did not affect AMX serum levels in dogs, indicating that the beta-lactamase was not released prior to AMX absorption. SYN-007 has the potential to expand microbiome protection via antibiotic inactivation to include 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 microflora and potentially lead to the outgrowth of pathogens like *Clostridium difficile*. SYN-004 (ribaxamase) [1,2] is a clinical stage, oral beta-lactamase enzyme therapy for use with IV beta-lactam antibiotics designed to preserve the gut microflora by degrading residual antibiotics in the GI tract [1]. A phase 2b study met its primary endpoint of significantly reducing *C. difficile* infection (CDI) in patients treated with ceftriaxone and ribaxamase.

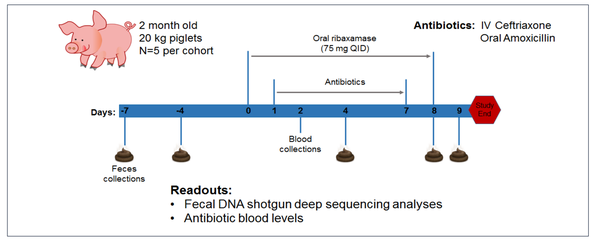
The ribaxamase clinical formulation consists of enteric-coated enzyme pellets engineered to protect the enzyme from stomach acid with enzyme release occuring at pH > 5.5, in the upper small intestine. As expected, use of the clinical formulation with oral amoxicillin in pigs prevented systemic absorption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

Novel formulations of ribaxamase, named SYN-007, intended to release enzyme in the GI tract at a site distal oral antibiotic absorption, were developed and evaluated *in vitro* and in pigs and dogs. Microbiome protection analyses based on whole genome shotgun sequencing of fecal DNA collected prior to and after antibiotic administration are in progress.

RESULTS

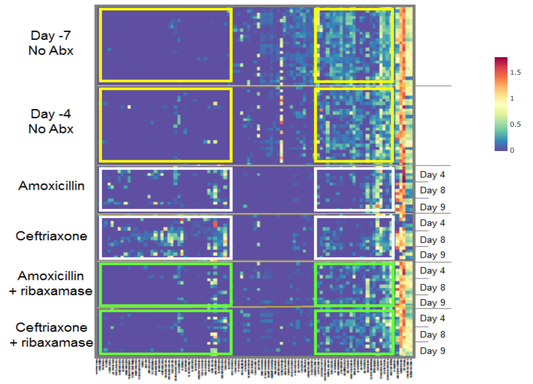
Porcine Model of Antibiotic-Mediated Gut Dysbiosis

A pig model of antibiotic-mediated dysbiosis was established. Normal pigs (20 kg, n=20) were treated with ceftriaxone (50 mg/kg, IV, SID), or oral amoxicillin (20 mg/kg, PO, BID) for 7 consecutive days. Cohorts (n=5) were also treated with ribaxamase (75 mg, PO, QID) for 9 days. Blood was collected on Day 2. Feces were collected at two pre-antibiotic time points (Days -7 and -4), during treatment (Day 4), and after antibiotics were stopped (Days 8 and 9). Fecal DNA was subjected to whole genome shotgun sequence metagenomic analyses.



Antibiotics Rapidly Disrupt the Gut Microbiome

Heat map analyses of the fecal microbial community based on species relative abundance. Each square represents a bacterial species present in individual animal microbiomes. Species are represented by the columns, and individual pig microbiome microbiota are displayed in rows. Yellow, white, and green boxes highlight changes in species abundance after antibiotic treatment.



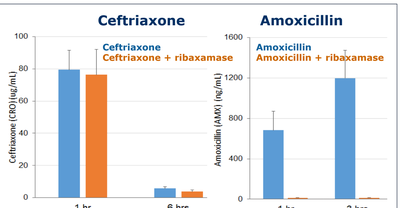
Comparison of the bacterial species present in the microbiomes of pigs that received the beta-lactam antibiotics (white boxes) to pretreatment (yellow boxes) revealed that antibiotic treatment caused the depletion of some species and the overgrowth of others. Amoxicillin and ceftriaxone-mediated microbiome changes were reduced in the presence of ribaxamase (green boxes).

Ribaxamase Protects the Microbiome and Does Not Affect CRO Serum Levels

Microbiomes prior to (Day -4) and after antibiotic administration (Day 8) were compared using a Dirichlet-Multinomial model likelihood ratio test [3]. Serum was collected on Day 2 of antibiotic delivery and ceftriaxone (CRO) and amoxicillin (AMX) levels were assessed. Data: mean ± SD.

Likelihood Ratio Test (Day -4 vs Day 8)

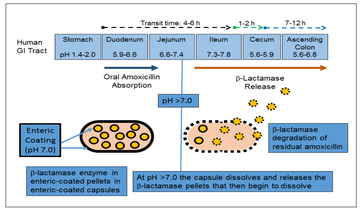
Treatment Group	Chi squared	P value
Ceftriaxone	469.93	<0.0001
Ceftriaxone + ribaxamase	79.22	0.38
Amoxicillin	720.56	<0.0001
Amoxicillin + ribaxamase	102.64	0.13



Each antibiotic caused dysbiosis as the microbiomes prior to antibiotic exposure were significantly different from the microbiomes after antibiotic treatment ($p<0.0001$). In contrast, ribaxamase prevented ceftriaxone- and amoxicillin-mediated dysbiosis, as the microbiomes before and after antibiotic exposure in the presence of ribaxamase were not significantly different ($p=0.38$ and 0.13, respectively).

Ceftriaxone levels were not significantly different in the presence or absence of ribaxamase at 1 or 6 hrs (two-tailed Student's T-test, $p=0.76$ or $p=0.08$, respectively). In contrast, no amoxicillin was detected in the serum of animals that received ribaxamase suggesting that amoxicillin was degraded prior to being absorbed.

Distal Release Ribaxamase Formulations



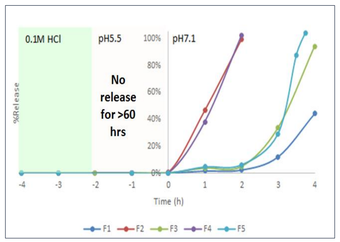
Five distal-release formulations (F1-F5) composed of enteric-coated pellets within enteric-coated capsules were tested *in vitro*. Capsules were held in 0.1M HCl for 2 hrs to simulate conditions in the stomach, and for 2->60 hrs at pH 5.5, conditions of the upper small intestine, and at pH 7.1, conditions of the lower small intestine/colon.

No enzyme release was detected in 0.1M HCl and at pH 5.5. Capsules remained intact for over 60 hrs at pH 5.5. At pH 7.1 two patterns of release were detected, a fast release that initiated upon exposure to pH 7.1 (F2, F4) and a slower release delayed for ~2 hrs after exposure to pH 7.1 (F1, F3, F5).

Early release of the beta-lactamase enzyme from the clinical ribaxamase formulation resulted in degradation of the orally-delivered amoxicillin in the GI tract prior to absorption.

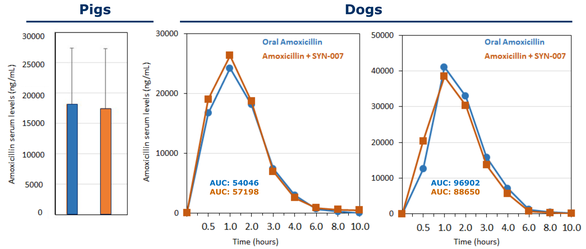
New formulations designed to be released later, in the distal small intestine, downstream of the site of amoxicillin absorption, were produced.

Dissolution under differing pH conditions



In Vivo Evaluation of SYN-007 Distal-Release Formulation

Formulation 2 (SYN-007) was chosen for evaluation in normal pigs (n=5 per cohort) or normal dogs (n=3). Pigs received oral amoxicillin (20 mg/kg) alone or with one capsule of SYN-007 (0.5 mg), anesthetized and bled 3 hrs later. Dogs received oral amoxicillin (80 mg/kg) alone and bled from 0.5 to 8.0 hrs later. Two weeks later, the same dogs received oral amoxicillin (80 mg/kg) plus one capsule of SYN-007 (10 mg). Animals were bled as before. Serum was analyzed for amoxicillin using a LC/MS/MS assay.



In pigs, there was no significant difference in amoxicillin serum levels with (orange) and without (blue) SYN-007 (two-tailed Student's T-test, $p=0.91$). For the dogs, PK curves for 2 of the 3 animals are displayed. The area under the curve (AUC) was calculated for each animal with amoxicillin alone (blue) and with amoxicillin + SYN-007 (orange). The AUCs were similar with and without SYN-007.

Fecal DNA whole genome shotgun metagenomics analyses are in progress to assess microbiome protection in the presence of SYN-007.

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
- Ribaxamase protects the gut microbiome from IV ceftriaxone in pigs
- SYN-007 is a new, distal-release formulation of ribaxamase for use with oral beta-lactam antibiotics
- SYN-007 did not interfere with oral amoxicillin absorption in pigs and dogs
- Microbiome analyses are in progress to assess protection of the gut microbiota with SYN-007

SYN-007 has the potential to protect the gut microbiome from oral beta-lactam antibiotics including amoxicillin without affecting antibiotic systemic absorption

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