Gut Infection Inactivated by Beta-Lactams is Intended to Prevent Microbiome Damage and Attenuate Antibiotic Resistance in Large Animal Models

Sheila Connelly1, Christian Furlan-Freguia2, Pooorni Subramanian3, Nur A. Hasan2, Rita R. Colwell1, Michael Kaleko1
1Synthetic Biologics, Inc., Rockville, MD, 2CosmosID, Inc., Rockville, MD

ABSTRACT

Background: Exposure of the gut microbiome to antibiotics can harm the microbe ecosystem, leading to antibiotic resistance. Beta-lactams are commonly used in the clinical stage, oral beta-lactamase enzyme intended to preserve the gut microbiome by protecting the gut from antibiotics without affecting antibiotic infection control efficacy. The use of ribaxamase for microbiota with oral antibiotics and synergy-dosed intestinal drug delivery was explored using two large animal models of antibiotic-mediated gut dysbiosis.

Methods: Ribaxamase was administered in the upper GI tract. New formulas were designed. S9N-007, were engineered to be released in the GI tract to oral antibiotic absorption. Ribaxamase and S9N-007 were evaluated in a pig model of antibiotic-mediated dysbiosis and S9N-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 (50 mg/kg, PO) –/- S9N-007 (10 mg, PO). Serum antibiotic levels were measured via LC/MS/MS and fecal DNA (16S) sequencing analyses were performed with CosmosID metagenomics software.

Results: In pigs, ribaxamase protected the gut microbiome from IV CRO and oral AMX and reduced antibiotic resistance genes. Ribaxamase had no effect on CRO serum levels. In contrast, AMX was not detected in the serum delivered with ribaxamase indicating that AMX was degraded in the GI tract prior to its absorption. Delivery of delayed-release S9N-007 with oral AMX in dogs did not affect AMX absorption, an AMX serum pharmacokinetics (PK) were similar with and without S9N-007.

Conclusions: Ribaxamase was completely degraded from damage caused by IV CRO and reduced emergence of antibiotic resistance genes. S9N-007, a novel formulation designed to target enzyme releasing 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. S9N-007 has the potential to expand microbiome protection via antibiotic inactivation to include oral as well as IV beta-lactam antibiotics.

BACKGROUND

In 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 outbreak of pathogens like Clostridium difficile. S9N-007 (ribaxamase) is a β-lactamase enzyme that is designed for use with IV β-lactam antibiotic designed to preserve the gut microbiota by degrading residual antibiotic. The β-lactamase is designed to be the only primary point of significantly reducing C. difficile infection (CDI) in patients treated with metronidazole.

The ribaxamase clinical formulation consists of enteric-coated enzyme pellets engineered to protect the enzyme from stomach enzymes and enteric-coated to ensure the pH at 5.5, in the upper small intestine. As expected, the use of the clinical formulation with ribaxamase was prevented systemically administered antibiotic prevents due to degradation of the antibiotic prior to its absorption.

Novel formulations of ribaxamase, named S9N-007, intended to release enzyme in the GI tract at a site distal oral antibiotic absorption, were developed and evaluated. The approach involved selecting the precise point in the small intestine, sequencing, and targeting of the formulated β-lactamase enzyme, and the delivery of the formulated β-lactamase enzyme to the site of distal oral antibiotic absorption.

Comparison of the bacterial species present in the microorganisms of pigs that received the beta-lactam antibiotics (white boxes) to pretreatment (yellow boxes) revealed that antibiotic resistance was the depletion of specific species and the expansion of other. Amoxicillin and ceftriaxone-mediated microbial changes were reduced in the presence of ribaxamase (green boxes).

RESULTS

Porcine Model of Antibiotic-Mediated Gut Dysbiosis

A 7-day model of antibiotic-mediated gut dysbiosis was performed. Normal pigs (20 kg, ±2) were treated with ceftriaxone (50 mg/kg, IV, SID) or oral amoxicillin (20 mg/kg, PO, BID). Blood and fecal samples were collected (12 AMX-coated pellets (20 mg/kg) delivered 8 hrs after treatment (1)), and after antibiotics were stopped (Days 8 and 9). Fecal DNA was subjected to_whole genome shotgun de-metagenomic analyses.

Ribaxamase Protects the Microbiome and Does Not Affect CRO Serum Levels

A single dose model of antibiotic-mediated gut dysbiosis (Day 1) was performed with a Dirichlet–Multinomial model likelihood ratio test (3). Serum was collected on Day 2 of antibiotic delivery were assayed and amoxicillin (AMX) levels were calculated. Only ceftriaxone (CRO) was SID-

No antibiotics

Rifaximin

Lactobacillus acidophilus


do not affect CRO serum levels. One antibiotic-carrying community (pig, Day 2) were named for it.

Bacteroides thetaiotaomicron

Bacteroides vulgatus

Rifaximin + Rifaximin

Lactobacillus acidophilus


do not affect CRO serum levels. One antibiotic-carrying community (pig, Day 2) were named for it.

Bacteroides thetaiotaomicron

Bacteroides vulgatus

Rifaximin + Rifaximin

Lactobacillus acidophilus

Antibiotics Rapidly Inhibit the Gut Microbiome

Heat map analyses of the fecal microbial community based on species relative abundances. Each square represents a bacterial species present in individual animal. The green squares represent the abundance of each species in the treatment group. The red squares represent the abundance of each species in the control group. The yellow squares represent the abundance of each species in the normalization group. The blue squares represent the abundance of each species in the antibiotic group.

Distal Release Ribaxamase Formulations

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.

Fecal DNA whole genome shotgun metagenomic analyses are in progress to assess microbiome protection in the presence of S9N-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 compared to patients receiving IV ceftriaxone + placebo.

S9N-007 is a new, distal-release formulation of ribaxamase for use with oral beta-lactam antibiotics.

S9N-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 S9N-007.

S9N-007 has the potential to protect the gut microbiome from oral beta-lactam antibiotics including amoxicillin without affecting antibiotic systemic absorption.

REFERENCES

1. Laxminarayan R, et al. (2015). Does the Use of Antimicrobial Agents in Food Animals Pose a Threat to the Effectiveness of Antimicrobial Drugs for Human Medicine? PLoS Med 12(4): e1001766. doi: 10.1371/journal.pmed.1001766. Published May 12, 2015. Copyright: © 2015 Laxminarayan R, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.


4. Phillips LG, et al. (2016). Preclinical Evaluation of Ribaxamase Formulation in Large Animal Models. Antimicrobial Agents and Infectious Diseases 8/4: 335-344. doi: 10.1037/aai0000527. Published August 2016. Copyright: © 2016 American Society for Microbiology. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.