Gut Antibiotic Inactivation by Oral Beta-Lactamase Therapy Protects the Microbiome from IV and Oral Antibiotics and Reduces Resistance in Large Animal Dysbiosis Models

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ABSTRACT

Background: Exposure of the gut microbiota to antibiotics can damage the microbiome and decrease its protective function. SYN-007 (ribaxamase) is a clinical stage oral beta-lactamase antibiotic with the potential to protect against antibiotic damage, interfering with antibiotic-mediated dysbiosis. The ability to delay antibiotic release in the stomach allows for protection from the gut microbiota. In animal models, ribaxamase protected the gut microbiota from IV and oral antibiotics, preventing antibiotic-mediated dysbiosis. This study set out to evaluate the ability of delayed release ribaxamase formulations to protect the gut microbiome from oral antibiotics in large animals.

Methods: Delayed-release ribaxamase formulations, named SYN-007, were engineered for dissolution in the lower small intestine distal to the site of oral antibiotic absorption. Multiple SYN-007 formulations were evaluated in vitro and three chosen for in vivo testing. Each formulation was administered to swine at 15 mg/kg PO, TID, +/- SYN-007 (10 mg, PO, TID) for 5 days. Serum amoxicillin levels were measured using a validated LC-MS/MS method, and whole genome shotgun sequence analyses were performed with CosmosID metagenomics software.

Results: With one SYN-007 formulation, serum amoxicillin levels were significantly different in both the first and last doses, indicating that SYN-007 did not reduce the antibiotic prior to its systemic absorption. Exposure to SYN-007 did not result in loss of some bacterial species and overgrowth of others, and emergence and proliferation of antibiotic resistance genes. The genes included encoded beta-lactamase, efflux pumps, and genes providing resistance to a broad range of antibiotic classes. In the primary Phase I studies, both oral antibiotic absorption and antibiotic resistance were reduced.

Conclusions: SYN-007 diminished antibiotic-mediated dysbiosis and mitigated emergent and propagation of antibiotic resistance in the gut microbiome. In the future, ribaxamase-resistant strains will be engineered for preservation of the gut microbiota without opportunistic infections and antibiotic resistance. SYN-007 has the potential to expand microbial protection to include oral as well as IV beta-lactam antibiotics.

BACKGROUND

IV beta-lactam antibiotics, including cephalosporins, are excrated into the bile into the intestines where they can destroy the intestinal microbiota and potentially lead to overgrowth of pathogens like Clostridium difficile. SYN-007 (ribaxamase) is a clinical stage oral beta-lactamase therapy for use with IV beta-lactam antibiotics designed to preserve the gut microbiome by degrading residual antibiotics and protecting it. A phase 2b clinical study met its primary endpoint of significantly reducing c. difficile disease in patients treated with cefoxitin + ribaxamase.

The ribaxamase clinical formulation consists of enteric-coated enzyme pellets engineered to protect the gut microbiota from antibiotics and with enzyme release occurring 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 antibiotic, presumably due to degradation of the antibiotic prior to absorption.

Novel formulations of ribaxamase, named SYN-007, intended to release enteric coated ribaxamase in the GI tract, were designed. Ribaxamase antibiotic formulation was developed and evaluated in vitro and in dogs. One SYN-007 formulation did not interfere with the excretion of the gut microbiota from antibiotics and antibiotic collateral damage, maintaining microbiota diversity and reducing propagation of antibiotic resistance in dogs.

RESUL TS

Distal Release Ribaxamase Formulations

Dissolution Formulations Dissolution Under Different pH Conditions

SYN-007 Formulation 3 Does Not Affect Amoxicillin Systemic Absorption

At Day 1, amoxicillin serum levels were not significantly different in the presence of or absence of SYN-007. In contrast, at Day 6, amoxicillin levels were significantly reduced with SYN-007 formulations 1 and 2 (p<0.01), but were not significantly different with formulation 3 (p<0.0005), reducing antibiotic levels 2-fold.

SYN-007 Formulation 3 did not interfere significantly with amoxicillin systemic absorption.

SYN-007 Protects the Gut Microbiome from Amoxicillin

Rifaximamide comparing bacterial species and beta-lactamase genes present in microbiomes of dogs prior to and after amoxicillin+/− SYN-007 Formulation 3.

SYN-007 Protects the Gut Microbiome from Amoxicillin

Compared to pretreatment microbiomes, there was no significant difference in enteric-coated antibiotic-dosed microbiomes, but there was significant difference in the microbiomes of animals treated with amoxicillin alone. In the syndromic (blue and gray dots) and antibiotic-pretreatment microbiomes (blue and gray dots) clustered together with Amoxicillin + SYN-007 microbiomes (green dots), while 42% Amoxicillin alone microbiomes (gray dots) fell well outside the clusters.

SYN-007: Protected the gut microbiome from collateral damage caused by oral amoxicillin administration.

SYN-007 Protects the Gut Microbiome from Amoxicillin

SYN-007: Protects the gut microbiome from collateral damage caused by oral amoxicillin administration.

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

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 treated with IV cefoxitin + ribaxamase.
• SYN-007 is a new, distal-release formulation of ribaxamase for use with oral beta-lactam antibiotics.
• SYN-007 reduced emergence of antibiotic resistance genes in dogs.

DISCLOSURES

SC, CPP, and HK are employees of Synthetic Biologics, Inc. RRW is the founder of CosmosID, Inc., a New-York service provider engaged by Synthetic Biologics, Inc. and MR are employees of CosmosID, Inc. This work was supported by Synthetic Biologics, Inc.