EP0175
ePoster Session
Clostridium difficile: news on clinical epidemiology and novel approaches to therapy

SYN-004, a clinical-stage, orally delivered beta-lactamase therapy that protects the gut microbiome from IV antibiotics is also efficacious with oral antibiotics in a pig model

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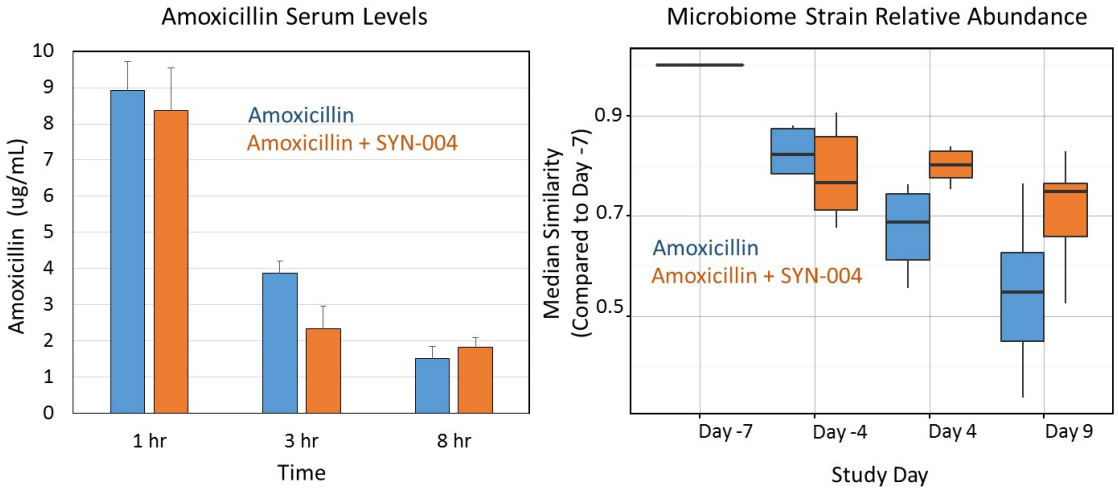
Background: Disruption of the intestinal microbiome is a major, unintended consequence of antibiotic exposure that can lead to overgrowth of pathogenic organisms such as *Clostridium difficile*. SYN-004 is an orally-delivered beta-lactamase for use with intravenous (IV) penicillins and cephalosporins to degrade residual antibiotics within the GI tract and thereby protect the microbiome. A Phase 2b study is in progress to assess the ability of SYN-004 to protect the microbiome and prevent antibiotic-associated diarrhea (AAD) and *C. difficile* infection (CDI) in patients receiving IV ceftriaxone (CRO). As SYN-004 has the potential to degrade orally-administered antibiotics in the GI tract prior to their absorption, a proof-of-concept study to evauate SYN-004 efficacy with oral amoxicillin was performed in normal pigs.

Material/methods: SYN-004 was manufactured in *E. coli* and formulated into enteric-coated pellets that release enzyme in the duodenum (at pH >5.5). To verify that SYN-004 effectively protected the microbiomes of pigs with IV antibiotics, normal piglets (~20 kg, n=5 per cohort) were treated with IV CRO (50 mg/kg QD for 7 days) with a separate cohort receiving CRO+SYN-004 (75 mg QID). In parallel cohorts, animals received oral amoxicillin (20 mg/kg BID for 7 days) or amoxicillin+SYN-004. Serum antibiotic levels were measured and whole genome shotgun sequence analyses of pig fecal DNA were performed.

Results: For CRO, serum levels were similar in the antibiotic-alone and antibiotic+SYN-004 cohorts indicating that SYN-004 did not alter systemic antibiotic levels. Microbiome analyses demonstrated that SYN-004 prevented CRO-mediated dysbiosis. Remarkably, for amoxicillin, serum levels were the same with or without SYN-004 treatment indicating that SYN-004 did not degrade the antibiotic prior to absorption. Microbiome analyses demonstrated that SYN-004 prevented amoxicillin-mediated loss of species diversity and protected the microbiome. A likelihood ratio test, performed using a parameterization of the Dirichlet-Multinomial distribution, compared the microbiomes from pretreatment day -4 with post-treatment day 9. For the amoxicillin-alone cohort, the p value was $1x10^{-9}$ indicating that the pre- and post-antibiotic fecal samples were significantly different. In contrast, for the amoxicillin+SYN-004 cohort, the p value was 0.997, indicating that the two fecal samples were not different.

Conclusions: SYN-004 protected the gut microflora in pigs from damage caused by IV CRO further supporting its clinical potential in humans. Notably, SYN-004 did not affect the absorption of orally-delivered amoxicillin and protected the microbiome from amoxicillin-induced dysbiosis. These data suggest that SYN-004 is released into the GI tract at a point distal to the absorption of amoxicillin but still proximal enough to degrade residual antibiotic. Therefore, SYN-004 has the potential to become the first therapy designed to protect the microbiome from antibiotics and prevent AAD and CDI. Importantly, the utility of SYN-004 can be expanded to include oral as well as IV antibiotics.


Amoxicillin Serum Levels
Amoxicillin (ug/mL)
10
9
8
7
6
5
4
3
2
1
0
Amoxicillin
Amoxicillin + SYN-004
1 hr
3 hr
8 hr
Time
Microbiome Strain Relative Abundance
Median Similarity (Compared to Day -7)
0.9
0.7
0.5
Amoxicillin
Amoxicillin + SYN-004
Day -7
Day -4
Day 4
Day 9
Study Day