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SYN-004, a Class A β-Lactamase Therapy for the Prevention of Antibiotic-Induced Disruption of Intestinal Microflora

M. Kaleko, J. A. Bristol, T. Parsley, S. Connelly, P. Koski; Synthetic Biologics, Inc., Rockville MD 20850

mkaleko@syntheticbiologics.com
240-238-3862

Abstract

Background: β-lactam antibiotics that are excreted into the intestine can damage the microflora, which can lead to serious infections such as *Clostridium difficile (C. diff)*. SYN-004 is a potent β-lactamase formulated for oral use with intravenous (IV) antibiotics to degrade antibiotics in the intestine.

Methods: SYN-004, formerly called P3A, was developed and evaluated by Ipsat Therapies, Helsinki, Finland. SYN-004 was engineered from the *Bacillus licheniformis* PenP enzyme to expand the hydrolysis of β-lactams to cephalosporins, including ceftriaxone (CRO), while maintaining its anti-penicillin activity. The use of CRO is a major risk factor for the development of *C. difficile* disease (CDI). Antibiotic hydrolysis was assessed *in vitro*. *In vivo,* SYN-004 activity was evaluated in the intestinal tract of jejunal-fistulated dogs (n=6) following administration of oral SYN-004 (0.44 mg/kg) and IV CRO (30 mg/kg).

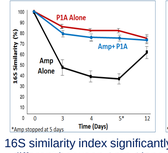
Results: *In vitro* antibiotic hydrolysis assays demonstrated that, compared to PenP, SYN-004 displayed improved degradation of cephalosporins: CRO, cefotaxime, cefazolin, cefoperazone, and cefuroxime. Activities against ampicillin and piperacillin were unchanged. Dog studies revealed that CRO was excreted at high levels into the intestine following IV delivery (mean C_{max} of 1500 μg/g of jejunal chyme), and a second CRO peak (mean 167 μg/g) was observed six hours later, after an additional feeding. Following delivery of SYN-004 ten minutes prior to IV CRO, the CRO concentration stayed low (≤5 μg/g chyme) for five hours in 4/6 dogs. The other two dogs did not eat prior to dosing and showed higher CRO concentrations at the early timepoints, presumably due to delayed gastric emptying. The second peak in CRO levels was not detected in any SYN-004-treated animal demonstrating that SYN-004 was present, remained functional, and hydrolyzed the CRO in the intestines of all treated dogs.

Conclusions: SYN-004 is a potent β-lactamase specifically engineered to hydrolyze an expanded range of antibiotics including the cephalosporins. Oral delivery of SYN-004 resulted in efficient degradation of intestinal ceftriaxone in dogs. Therefore, SYN-004 is a promising candidate to protect the intestinal microflora to prevent antibiotic-associated adventitious infections such as *C. diff*.

Background

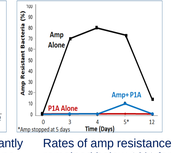
The β-lactam antibiotics excreted via the bile duct into the intestine can disrupt the intestinal microflora. In clinical trials, the β-lactamase, P1A, given orally with IV penicillins preserved the diversity of the intestinal microbiome, reduced the selection for antibiotic-resistant coliforms, efficiently degraded piperacillin/tazobactam in the intestine, and did not alter plasma antibiotic levels. However, P1A has limited utility as it does not efficiently degrade cephalosporins, use of which is a major risk factor for C. *difficile* infection.

Similarity Index



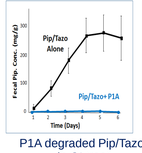
16S similarity index significantly different between Amp Alone and Amp+P1A

Amp-Resistant Bact.



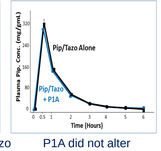
Rates of amp resistance remained below 10% for Amp+ P1A

GI Tract



P1A degraded Pip/Tazo in GI tract

Systemic



P1A did not alter antibiotic plasma levels

Therefore, SYN-004 was engineered from P1A by introducing a one amino acid change, D276N. SYN-004 was characterized *in vitro*, and proof of concept for the use of SYN-004 to degrade the cephalosporin, ceftriaxone, in the intestinal tract of dogs was achieved. SYN-004 GMP manufacturing is in progress and a clinical trial is expected in 2014.

Results

SYN-004 Antibiotic Degradation Kinetics

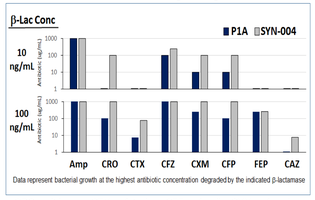
SYN-004 was compared directly to its parent enzyme, P1A. Michaelis-Menten kinetics were determined using non-linear regression analyses. Relative antibiotic hydrolysis was evaluated with a microtiter plate activity assay using E. *coli* growth as the read-out for antibiotic degradation.

Kinetics

Antibiotic	P1A		SYN-004 (D276N)	
	K_m	k_{cat}	K_m	k_{cat}
	μM	s^{-1}	μM	s^{-1}
Ampicillin	157	3,369	161	2,160
Piperacillin	49	939	53	816
Ceftriaxone	400	0.05	38	83
Cefotaxime	363	246	213	36
Cefazolin	22	93	37	192
Cefuroxime	107	233	277	35
Cefoperazone	7	10	2	17
Cefepime	n/a*	n/a	1,357	133
Ceftazidime	n/a	n/a	1,505	274

n/a*-Not active

Antibiotic Hydrolysis



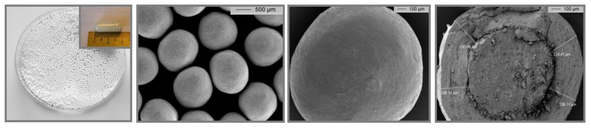
CRO: ceftriaxone, CTX: cefotaxime, CFZ: cefazolin, CXM: cefuroxime, CFP: cefoperazone, FEP: cefepime, CAZ: ceftazidime

SYN-004 displayed enhanced activity against ceftriaxone, cefotaxime, cefuroxime, cefoperazone, and ceftazidime while retaining activity against the penicillins.

Manufacturing

SYN-004 was manufactured in E. *coli*. High yields of 14 g/L were obtained. A simple, three step purification process was developed with final yields of ~45% with >95% purity. For oral delivery, SYN-004 was formulated into enteric-coated pellets that were used to fill capsules. The enteric coating remained intact at low stomach pH and dissolution occurred at pHs > 5.5, the pH levels found in the duodenum. The released SYN-004 is expected to degrade antibiotics excreted via the bile in the small intestine, thus protecting the intestinal microbiome.

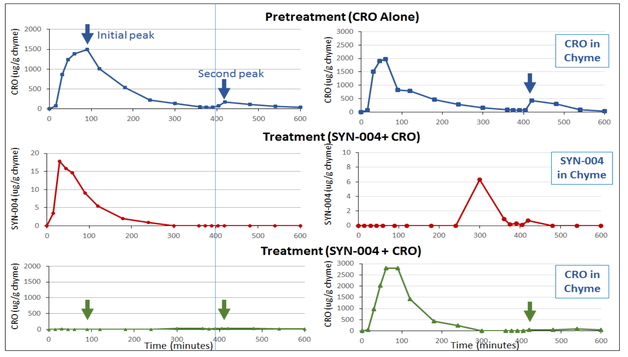
SYN-004 Enteric-Coated Pellets



SYN-004 enteric-coated pellets were uniform spheres of ~1 mm diameter. The pellets displayed a smooth appearance without noticeable cracks demonstrating that the enteric coating was intact. The cross-section of a single pellet displays the sucrose core, SYN-004 drug product, and the enteric coating layers.

SYN-004 Degraded Ceftriaxone in the GI Tract of Dogs

SYN-004 was tested in the intestinal tract of jejunal-fistulated dogs (n=6) following oral delivery of SYN-004 enteric-coated pellets (0.44 mg/kg) and IV ceftriaxone (30 mg/kg).

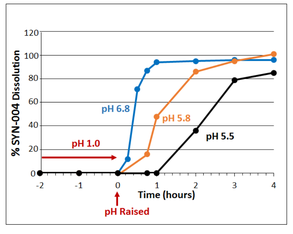


The dog studies revealed that ceftriaxone (CRO) was excreted at high levels into the intestine following IV delivery (mean C_{max} of 1500 μg/g of jejunal chyme), and a second CRO peak (mean 167 μg/g) was observed six hours later, after an additional feeding. When SYN-004 was delivered orally 10 min prior to IV CRO, the CRO concentration stayed low (< 5 μg/g chyme) for five hours in 4/6 dogs (left panels). The other dogs did not eat prior to dosing and displayed a higher CRO concentration at the early time points, presumably due to delayed gastric emptying (right panels). Importantly, the second peak in CRO levels was not detected in any SYN-004-treated animal demonstrating that SYN-004 was present, remained functional, and hydrolyzed the CRO in the intestines of all treated dogs.

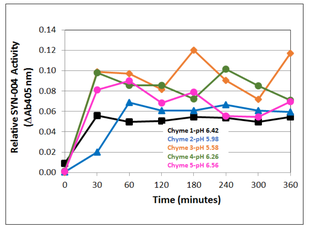
In Vitro SYN-004 Pellet Dissolution and Stability in Human Chyme

SYN-004 pellets were held in a 0.1 N HCL solution for 2 hrs followed by incubation in buffers at pHs 6.8, 5.8, or 5.5 from 0.25 to 4 hrs. Human chyme from five different donors was characterized based on pH, liquid content, and protease activities. SYN-004 pellets were incubated in each chyme from 30 to 360 min. All samples were assessed for SYN-004 activity using a CENTA chromatogenic assay.

pH Dissolution Profile of SYN-004 Pellets



Stability of SYN-004 Pellets in Human Chyme



SYN-004 pellets were protected at low pH while dissolution occurred at pHs > 5.5, with pHs 6.8 and 5.8 showing more rapid dissolution than pH 5.5. In human chyme, SYN-004 pellets showed rapid dissolution, within 30-60 min. High-level SYN-004 activity was observed for at least 6 hours, demonstrating SYN-004 enzyme stability in human chyme.

Conclusions

- SYN-004 differs from its parent enzyme, P1A, by one aa, D276N
- SYN-004 displayed improved degradation of a panel of cephalosporins, including ceftriaxone
- Manufacturing of SYN-004 was optimized to obtain high yields with simplified purification
- Enteric-coated SYN-004 pellets are inert at low pH and rapidly dissolute at pHs >5.5
- Enteric-coated SYN-004 pellets rapidly dissolute in human chyme and display stable activity for at least 4 hours
- In dogs, oral delivery of SYN-004 pellets resulted in efficient degradation of intestinal CRO
- Clinical evaluation of SYN-004 is anticipated in to begin in late 2014

References

1. Harmoinen, J, Vaali, K, Koski, P, Syrjanen, K, Laitinen, O, Lindevall, K, Westemarck, E. (2003). Enzymatic degradation of a beta-lactam antibiotic, ampicillin, in the gut: a novel treatment modality. J. Antimicrob. Chemother. 51:361-365.
2. Pitout, JD. (2009). IPSAT P1A, a class A beta-lactamase therapy for the prevention of penicillin-induced disruption to the intestinal microflora. Curr. Opin. Investig. Drugs. 10:838-844.