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

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Abstract

Background: Bluntant antibiotics that are excreted into the intestine can damage the microbiome, which can lead to serious effects such as Clostridium difficile colitis. SYN-004 is a β-lactamase formulated for oral use with an engineered β-lactamase to degrade antibiotics in the stomach and small intestine.

Methods: SYN-004, formerly called PTA, was developed and evaluated by Therapeutics, Inc. (now Synthetic Biologics, Inc.) using their proprietary β-lactamase technology to degrade specific antibiotics in the GI Tract. SYN-004 was delivered in enteric coated pellets (mean diameter of 3-4 mm), thus allowing for timed release function in the intestine. SYN-004 is inert, pH-activated, and extends antibiotic refractory time in the GI Tract.

Results: SYN-004 represents a novel approach to oral delivery of antibiotics. SYN-004 displayed enhanced activity against ceftriaxone, cefotaxime, cefuroxime, ceftepime, and cefetamet while retaining activity against the penicillins. SYN-004 demonstrated enhanced activity against ceftriaxone, cefotaxime, cefuroxime, ceftepime, and cefetamet while retaining activity against the penicillins.

Conclusions: SYN-004 differs from its parent enzyme, P1A, by one aa, D276N.

SYN-004 showed improved degradation of cep胎ins compared to the class A enzyme.

Manufacturing

SYN-004 was manufactured in E. coli. High yields of 14 g/L were obtained. A single, step purification process was developed with final yields of >90% with >95% purity. For oral delivery, SYN-004 was formulated into enteric-coated pellets that we used to fill capsules. The enteric coating remained intact at low stomach pH and dissolution occurred at pH > 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.

Conclusion

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

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 cephalosporins, cefuroxime, in the intestinal tract of dogs was achieved. SYN-004 GMP manufacturing is in progress and a clinical trial is expected in 2014.

SYN-004 Antibiotic Degradation Kinetics

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

SYN-004 Degrade 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 Cmax of 1590 μg/g of jejunal chyme), and a second CRO peak (mean 167 μg/g) was observed six hours later, following an additional feeding. When SYN-004 was delivered orally 10 min prior to IV CRO, the CRO concentration stayed low (<5 μg/g) for five hours, similar to the control dogs (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 and functional, and hydrolyzed the CRO in the intestines of all treated dogs.

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 dissolve at pHs >5.5.
• Enteric-coated SYN-004 pellets rapidly dissolve 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 to begin in late 2014.

References


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