Development of Therapeutic Agents that Protect the Colonic Microflora from Beta-Lactam Antibiotics for the Prevention of Clostridium difficile Infection

S. Connelly1, T. Parsley2, P. Koski3, M. Kaleko1; 1Synthetic Biosciences, Inc., 2SynPhaGen, LLC, Rockville MD

Abstract

Background

IV beta-lactam antibiotics, including cephalosporins, are excreted via the bile duct into the intestine where they can disrupt the intestinal microflora and potentially lead to the outgrowth of pathogenic like Clostridium difficile. SYN-004 is a clinical stage, oral beta-lactamase enzyme therapy for use with IV beta-lactam antibiotics to preserve the gut microflora by degrading residual antibiotics in the intestine. The intended indications are prevention of Clostridium difficile infection and antibiotic-associated diarrhea. Phase I clinical studies evaluated SYN-004 for safety and measured a single dose of up to 750 mg and multiple doses of 300 mg q.i.d. for 7 days. SYN-004 was neither systemically bioavailable nor immunogenic in humans. A Phase 2a clinical study of SYN-004 was initiated in Q1 2015 and a Phase 2b clinical study is expected to begin in 2H 2015.

SYN-004 preclinical and clinical data are being presented in Poster 953. While SYN-004 degrades penicillins and cephalosporins, it does not inactivate cephalosporins. To expand this prophylactic approach to all beta-lactam antibiotics, SYN-004 has the potential to develop product candidates from three broad spectrum cephalosporins, P2A, NDM-1, and KPC-1. P2A was derived from Bacillus cereus.

Protein expression in E. coli

Over 100 different E. coli production strains for P2A, NDM, and KPC were evaluated for expression via SDS/PAGE and activity using the CENTA chromogenic assay. P2A and NDM are class B meta-lactams that require zinc for activity. P2A and NDM were expressed and with zinc in the bacterial growth media. For the meta-lactams, P2A and NDM, the addition of zinc to the bacterial growth media was found to shift expression from inclusion bodies to the soluble fractions. The highest expression levels for each enzyme were chosen for 5X bioreactor fermentation and chromatographic purification (95%). Final yields for each were ~600 mgL. A recombinant assay using E. coli growth as the model for antibiotic degradation was used to assess the potentials of each beta-lactam with 18 antibiotics. A total of 10 to 100 µg of each antibiotic was mixed with 10 or 100 mg of each enzyme and incubated for 1 hour at 37°C. The incubation was quenched by adding SDS, and the remaining antibiotic was measured by SDS/PAGE and activity using the CENTA chromogenic assay. For the meta-lactams, P2A, NDM, and KPC all displayed broad antibiotic degradation profiles that included carbapenems, NDM was the most potent beta-lactama and very degraded all tested cephalosporins and carbapenems. NDM and P2A were resistant to the inhibitors sulbactam, tazobactam, and adjunct KPC was the only beta-lactama with activity against the monobactam, aztreonam. P2A-related biological activity in human chyme for at least 6 h.

These data indicate that all three beta-lactama enzymes can be manufactured and have sufficient potency to be developed into oral therapeutics. Each has the potential to protect the gut microbiome from most beta-lactam antibiotics and provide prophylaxis for Clostridium difficile infection and antibiotic-associated diarrhea.

Antibiotic Degradation Kinetics

The purified beta-lactama enzymes were assessed for antibiotic hydrolysis activity with a microtiter plate assay activity using E. coli growth as the read-out for antibiotic inactivation. A total of 10 to 1000 nM of each enzyme, E. coli growth and added antibiotic was paired to quantify the hydrolysis of the antibiotic. The graph displays the highest antibiotic concentration that allowed bacterial growth, indicating antibiotic inactivation.

Results

Conclusions

Three broad-spectrum beta-lactamas, P2A, NDM, and KPC, were manufactured and purified with retention of biological activity. P2A, NDM, and KPC displayed antibiotic hydrolysis activity on a wide range of beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems. The metallo-enzymes, P2A and NDM, were resistant to the beta-lactam inhibitors sulbactam, tazobactam, and aztreonam. P2A displayed stability in human chyme. All three carbapenemas have the potential to be developed into oral therapeutics to protect the gut microbiome from most beta-lactam antibiotics and to prevent Clostridium difficile infection and antibiotic-associated diarrhea.

S. Connelly and T. Parsley. SYN-004 preclinical and clinical data are being presented in Poster 953.