Toxicology and Applied Pharmacology 374 (2019) 32–40

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Preclinical evaluation of the maximum tolerated dose and toxicokinetics of enteric-coated lantibiotic OG253 capsules☆

N.V. Rajeshkumar[a,1], Johan A. Kers[b,1,2], Scott Moncrief[a], Anthony W. Defusco[c,3], Jae H. Park[c], Martin Handfield[c,*]

[a] Intrexon Corporation, Human Therapeutics Division, 20358 Seneca Meadows Pkwy, Germantown, MD 20876, USA
[b] Intrexon Corporation, Industrial Products Division, 29 Oyster Point Blvd., South San Francisco, CA 94080, USA
[c] Oragenics, Inc., 13700 Progress Blvd., Alachua, FL 32608, USA

ARTICLE INFO

Keywords:
CDAD
Mutacin
OG253
Lantibiotic
Lanthipeptide
Bacteriocin
Nisin and Toxicokinetics

ABSTRACT

Clostridium difficile associated disease (CDAD) is the leading infectious cause of antibiotic-associated diarrhea and colitis in the United States. Both the incidence and severity of CDAD have been increased over the past two decades. We evaluated the maximum tolerated dose (MTD) and toxicokinetics of OG253, a novel lantibiotic in development for the treatment of CDAD. OG253 was orally administered to Wistar Han rats as enteric-coated capsules in a one-day dose escalation study, followed by a seven-day repeated dose toxicokinetics study. All three doses of OG253 (6.75, 27 and 108 mg/day) were generally well-tolerated with no treatment-related clinical signs, alterations in body weight or food consumption in both one-day acute tolerability and seven-days repeated dose tolerability and toxicokinetics study. OG253 capsule administration neither significantly alter the weight of organs nor affect the hematology, coagulation, clinical biochemistry parameters and urine pH compared to placebo capsule administered rats. LC-MS/MS analysis did not detect OG253 in the plasma, indicating that OG253 is not absorbed into the blood from the rat gastrointestinal tract. Glandular atrophy of the rectal mucosa was noticed in two out of six rats administered with a high dose of OG253. Surprisingly, we found that OG253 treatment significantly lowered both serum cholesterol and triglyceride levels in both sexes of rats. Overall, there was a 29.8 and 61.38% decrease in the serum cholesterol and triglyceride levels, respectively as compared to placebo-treated rats. The well-tolerated high dose of OG253 (425.7 mg/kg/day) is recommended as the MTD for safety and efficacy studies. Further preclinical study is needed to evaluate the safety profile of OG253 under longer exposure.

1. Introduction

Clostridium difficile (*C. difficile*), a spore-forming gram-positive bacteria, is currently the most frequently identified pathogen causing antibiotic-associated diarrhea and the main cause of nosocomial diarrhea. *C. difficile* associated disease (CDAD) is a global public health challenge where even mild to moderate infections can quickly progress to a fatal disease if not treated promptly (Leffler and Lamont, 2015; Lessa et al., 2015). While most cases of CDAD are correlated with antibiotic use and hospitalization, CDAD can also occur in patients without exposure to antibiotics. *C. difficile* is commonly acquired during hospital stays (healthcare-acquired infections), infecting approximately 1% of patients admitted to hospitals in the United States (Magill et al., 2014; Leffler and Lamont, 2015; Di Bella et al., 2016; Martin et al., 2016; Ofosu, 2016). The prevalence of CDAD has been increasing globally over the last 5 years, particularly in elderly populations. The increase in CDAD incidence has been largely attributed to an increasing frequency of emergence and spread of more virulent strains. *C. difficile* has become the most common cause of health care-associated infections in U.S. hospitals, and the excess health care costs related to CDAD are estimated to be as much as $4.8 billion for acute care facilities alone (Magill et al., 2014).

☆ Note: Presented in part at the American Society for Microbiology (ASM) Conference on Antibacterial Development held in Washington, DC, on Dec. 11–14, 2016.

* Corresponding author.
E-mail address: mhandfield@oragenics.com (M. Handfield).

[1] Contributed equally to this work.
[2] Current affiliation: AbSci LLC, 101 E 6th Street, Suite 300, Vancouver, WA 98660, USA.
[3] Current affiliation: Brammer Bio, LLC. 13,859 Progress Blvd., Alachua, FL 32615, USA.

https://doi.org/10.1016/j.taap.2019.04.019
Received 9 November 2018; Received in revised form 22 April 2019; Accepted 25 April 2019
Available online 26 April 2019
0041-008X/

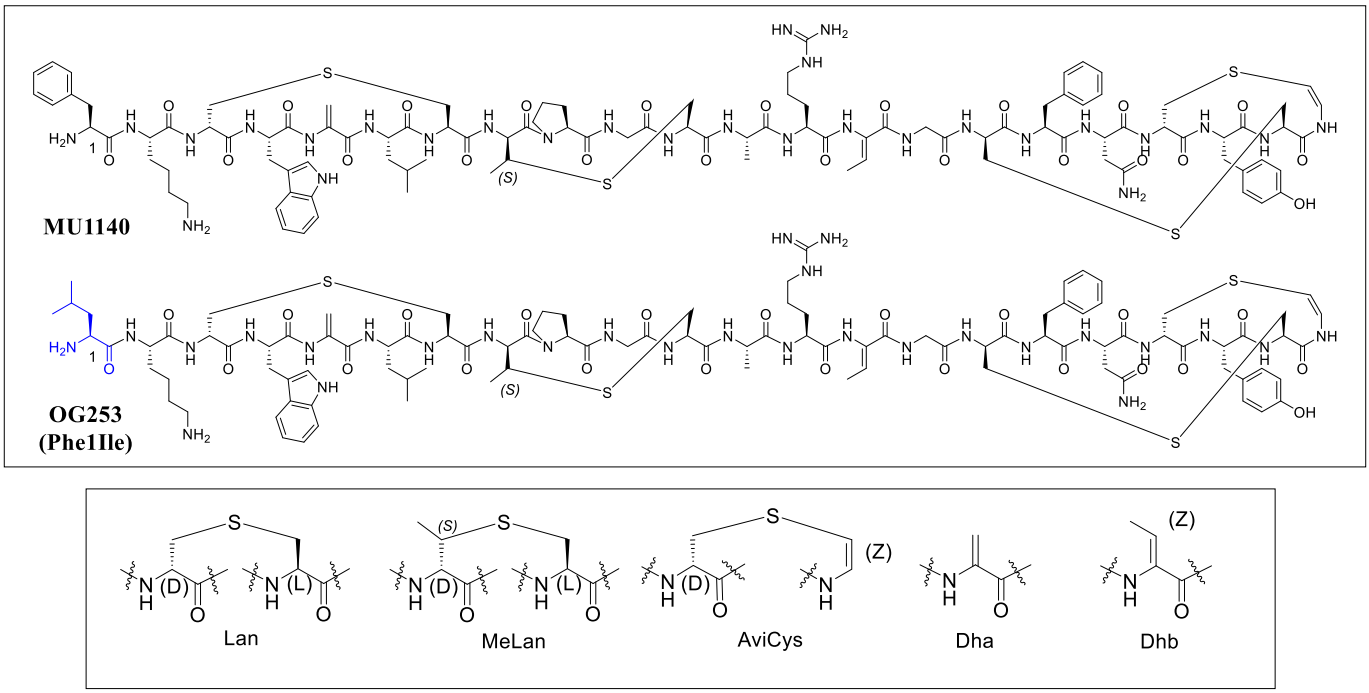

Fig. 1. Structures of MU1140 and OG253. The structure of MU1140 and OG253 (top panel). Amino acid substitution from MU1140 is highlighted in blue font color. Post-translationally modified residues in lantibiotics are shown in the bottom panel. Abbreviations: AviCys, aminovinyl-*D*-cysteine; Dha, 2,3-didehydroalanine; Dhb, 2,3 didehydrobutyrine; Lan, lanthionine; MeLan, methyl-lanthionine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Lantibiotics are cationic polycyclic lanthipeptides that derive their names from the thioether ring containing amino acids lanthionine (Lan, Ala-S-Ala) and/or 3-methyl-lanthionine (MeLan, Abu-S-Ala). This class of compounds is generally produced by gram-positive bacteria and often include other post-translationally unsaturated amino acids such as 2,3-didehydroalanine (Dha), 2,3-didehydrobutyrine (Dhb), and the unsaturated lanthionine derivatives aminovinyl-*D*-cysteine (AviCys) at their *C*-terminus (Fig. 1) (Chatterjee et al., 2005; Smith et al., 2008; Ross and Vederas, 2011; Theuretzbacher et al., 2019). To date, only a few lantibiotics has been evaluated in clinical studies. However, no lantibiotics have been approved by regulatory authorities for CDAD (Boakes et al., 2016; Ongey et al., 2017). This is likely due to economic and technical challenges related to the scale-up manufacturing of lantibiotic quantities for human testing. The lantibiotic NVB302, an actagardine derivative, was specifically developed and tested for the treatment of *C. difficile* infections. NVB302 completed a Phase-1 clinical trial (Novacta Biosystems Limited, Hertfordshire, UK) (Sandiford, 2015). The pre-clinical and clinical data available for NVB302 (MJ Dawson, Novacta Biosystems, personal communication), duramycin (Grasemann et al., 2007) and other lantibiotics reported to date supports the notion that lantibiotics, as a class, are efficacious and well tolerated in humans (Smith et al., 2008).

Mutacin 1140 (MU1140) is a lantibiotic that is naturally produced by *Streptococcus mutans* (Hillman et al., 1984; Sahl et al., 1995; Hillman et al., 1998). Since it was discovered nearly 34 years ago, this compound has received considerable attention as a novel antimicrobial peptide because of its spectrum of activity, potency, low frequency of antimicrobial resistance, limited cytotoxicity and overall pharmacological profile (Ghobrial et al., 2009; Ghobrial et al., 2010). While several other groups have investigated the structure-function of key residues of MU1140 (Chen et al., 2013) and other archetype lantibiotics (Ross and Vederas, 2011; Knerr and van der Donk, 2012), our group used saturation mutagenesis as a means to derive comprehensive structure-function data of every natural amino acid substitution along the sequence of the core peptide in an unbiased and addressable fashion (Kers et al., 2019). From those studies, the blueprints enabling further development of "druggable" MU1140-variants, based on their improved pharmaceutical, therapeutic and physicochemical properties, emerged. The lead compound OG253 (MU1140-Phe1Ile) was identified as a potential drug candidate from the first-generation single-substitution variants of MU1140, based on several *in vitro* assays used to select the top performing compounds. Several assays including a) potency testing (MIC against *Micrococcus luteus*, *C. difficile*, Vancomycin-Resistant Enterococci (VRE), *Staphylococcus aureus*, *Streptococcus pneumonia*, *Mycobacter phlei* and *Pseudomonas aeruginosa*; b) cytotoxicity screening on HepG2 hepatocytes; c) *in vitro* pharmacological profiling with the Safety Screen 44™; d) metabolic and chemical stability in biologically-relevant fluids (fasted state simulated gastric fluid, fasted state simulated intestinal fluid and serum); and e) characteristics in common formulations were used for the identification of OG253 as a lead compound. OG253 was characterized by a half-life in simulated gastric fluid > 240 min. The stability of OG253 in simulated intestinal fluids was much lower (95 min). The half-life of OG253 in serum was 270 min. The top performers in the *in vitro* assays were ultimately tested for their efficacy *in vivo* in the Syrian hamster CDAD animal model. The *in vitro* serum assay data set correlated best with the *in vivo* efficacy study results. It is noteworthy to mention that the animals were cannulated in these initial studies to assure delivery of OG253 to the distal portion of the intestine and circumvent the known proteolytic sensitivities of this lanthipeptide. Of particular interest, OG253-treated animals did not experience characteristic CDAD relapse following initial resolution of their CDAD infections over the 21-days observation period in this model (Kers et al., 2018a; Kers et al., 2018b). To circumvent the known trypsin/chymotrypsin proteolytic issues involving OG253, we sought to develop an encapsulated, enteric-coated D-mannitol-formulation that would target capsule release to the distal portion of the intestine.

The objective of the present study was to evaluate the repeated dose toxicokinetics and any potential toxicity of OG253 capsules, following daily (TID) oral administrations of three different doses capsules (6.75, 27 and 108 mg/day) for a single day or 7-day dosing (7 consecutive

days) in rats. The animals were observed for potential signs of toxicities using clinical observations, as well as an extensive series of tests including hematology, coagulation, clinical chemistry, urinalysis, macroscopic examination, histopathological evaluation, and clinical chemistry. The bioavailability of OG253 in plasma was determined by LC-MS/MS analysis. Overall, our results indicate that OG253 is safe and may serve as a lead compound for further preclinical analyses and development.

2. Materials and methods

2.1. Drug manufacturing, encapsulation, and characterization

OG253 was manufactured at the gram scale using proprietary methods (Oragenics, unpublished). Briefly, fermentations were performed to produce crude peptide in 200 L bioreactors using fed-batch fermentations under aerobic stirred-tank conditions with automated temperature/pH/dissolved oxygen controls. Purifications of crude peptide were carried out using conventional column chromatography with 0.1% trifluoroacetic acid (TFA) containing buffers (Oragenics, unpublished). Purified fractions were lyophilized, resulting in a trifluoroacetate salt of the lanthipeptide. The purity, quantity and identity of each compound during manufacturing was determined by HPLC and LC/MS analysis as previously reported (Kers et al., 2018a; Kers et al., 2018b; Kers et al., 2019). Briefly, Waters XBridge C18 column was used, with particle size 3.5 μm, 3.0 × 150 mm. Buffer A was 0.05% TFA in H_2O and buffer B was 0.05% TFA and 5% H_2O in acetonitrile (ACN). The gradient was 2% buffer B to 85% buffer B over 38 min at a flow rate of 0.6 mL/min. Injection volume was 50 μL. The mass of peak of interest was analyzed by electrospray ionization-Time of Flight (ESI-TOF) mass spectrometry with a desolvation temperature of 200 °C and an ion source temperature of 100 °C. Capillary Voltage and sample cone voltage were 3000 V and 80 V, respectively. NMR spectra of OG253 were acquired and analyzed as previously reported (Kers et al., 2019). The spectra were acquired on an Agilent NMRS instrument, operating at 600 MHz for proton, and equipped with a high-temperature superconductor (HTS) 1.5 mm probe (University of Florida NMR Core Facility, Gainesville, Florida). The water signal was suppressed in a wet1D experiment. The precedent parameters were used to set up a) a Total Correlation Spectroscopy (TOCSY) experiment with a mixing time of 150 ms, b) a Rotating-Frame NOE Spectroscopy (ROESY) experiment with a mixing time of 200 ms, c) a Heteronuclear Single Quantum Coherence (HSQC) experiments optimized for a one-bond coupling constant of 146 Hz and d) a Heteronuclear Multiple Bond Correlation (HMBC) experiments optimized for a long-range coupling constant of 8 Hz. A 5 mg sample of OG253 was dissolved in 60 μL of a mixture deuterated acetonitrile:water, 3:1, yielding a 37 mM solution. The ^{19}F spectrum of the sample was run in the same 1.5 mm tube, on a Varian Inova spectrometer operating at 500 MHz for proton, and equipped with a 5 mm probe. Peptide International (Louisville, KY, USA) performed elemental Analysis.

Size-9 gelatin capsules (Torpac, Fairfield, NJ, USA) were manually filled to the appropriate capacity, sealed using dyed hydroxypropyl methylcellulose (HPMC, aka Pharmacoat 606, Shin Etsu Chemical, Wiesbaden, Germany, EU), and enteric-coated using an Eudragit-based proprietary formulation (Emerson Resources, Norristown, PA, USA). The enteric-coating assured a capsule delivery that could withstand the transit time in the stomach (~pH 1–5) but dissolve at ~pH 7, releasing OG253 in the distal portion of the small intestine of hamsters (Kers et al., 2018b). The banding solutions were prepared by mixing 16.0% (w/w) HPMC, 0.5% of dye (w/w), 33.1% of deionized H_2O (w/w) with 50.4% of dehydrated USP ethanol (w/w). Photograph representative of color-coded placebo and OG253 capsules are shown in Fig. 2. Three different doses of OG253 in enteric-coated capsules were administered orally three times a day (TID) at 8 h apart. The doses were 26.6 mg/kg/day (low dose), 106 mg/kg/day (mid dose) and 425.7 mg/kg/day (high dose). Dyes used in coloring included Tartrazine (yellow), Allura Red, Fast Green FCF (Sigma) or FD&C Blue # 1 (Spectrum Chemicals). D-mannitol was used as an excipient and bulking agent when appropriate. Release-testing parameters included a) weight variation (Pharmacopeia., 2012), b) content uniformity and assay (Pharmacopeia., 2011), measuring concentration & purity by RP-HPLC (Kers et al., 2019) (c) activity by minimum inhibitory concentration (MIC) determination against *M. luteus* (Kers et al., 2019) and d) disintegration testing (Pharmacopeia, 2012). For the latter, coated capsules were successively submerged in simulated gastric fluid (SGF) with pepsin for 1 h (pH 1.2), followed by transfer to simulated intestinal fluid (SIF) with pancreatin (pH 6.8) for 1 h.

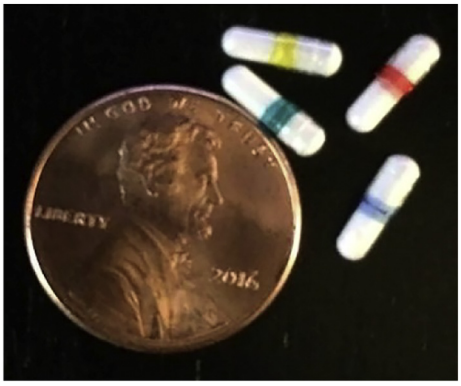

Fig. 2. Representative images of enteric-coated size 9 capsules used for the study. Blue-banded capsules were used as Placebo capsules. Purple-banded capsules were used for OG253 low dose. Green-banded capsules were used for OG253 mid dose. Red-banded capsules were used for OG253 high dose. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Animal studies

Wistar Han rats (Envigo Research Model and Services, Frederick, MD) were used for the study. 8–10 weeks of age rats were randomly assigned to groups based body on weight and/or sex. This strain of rat has been historically used in safety evaluation studies and is recommended by appropriate regulatory agencies (Gauvin et al., 2019). The numbers of animals, study design, and treatment of animals were reviewed, and approved by the Institutional Animal Care and Use Committee (IACUC). Enteric-coated color-coded capsules were administered *via* oral gavage. After capsule administration, animals were administered 250 μL of water (pH 4.0) *via* oral gavage using a dosing syringe. Animal welfare was in compliance with Intrexon's IACUC and the Guidelines for the Care and Use of Laboratory Animals and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, Office of Laboratory Animal Welfare, Department of Health and Human Services. Rats were individually housed in polycarbonate cages. Unless noted otherwise, rats were maintained under standard animal housing conditions, with free access to food and water and a 12 h light/12 h dark cycle. Animals were housed under specific pathogen free (SPF) conditions in a room, which is controlled and continuously monitored at a temperature of 72 °F ± 8 °F and 30–70% relative humidity by an automated environmental monitoring system. 8–0 weeks old rats were used for the study. Cages were bedded with fresh shredded paper bedding (Tek Fresh Bedding) from Envigo Research Model and Services (Frederick, MD) or Iso-Pad enrichment bedding if needed. All caging components including cages, wire bar, and water bottles were steam sterilized before use.

Table 1
1-day tolerability study design of rats orally gavaged with OG253 capsules.

Group	N and sex	Dose	OG253 per capsule (mg)	Number of capsules/dose	Dosing interval	Daily dose (mg)	Daily dose (mg/kg)
1	4 M	OG253 low dose	2.25	1	8 hourly (TID)	6.75 mg	26.6
2	4 M	OG253 mid dose	9	1	8 hourly (TID)	27 mg	106
3	4 M	OG253 high dose	18	2	8 hourly (TID)	108 mg	425.7

After overnight fasting, rats were administered with OG253 capsules three times per day (TID, at intervals of 8 h).
All animals were observed for clinical signs including mortality and morbidity, immediately
following the dosage, 1, 2, 4, 8, and 12 h post dosage and twice daily for a total of 5 consecutive days. Body weights were measured daily. Food consumption was recorded daily. Animals were sacrificed on day 5.
M denotes male rats.

2.3. One-day acute tolerability

Twelve male Wistar Han rats were randomly assigned to 3 study groups ($N = 4$ each) based body weight as outlined in Table 1. After overnight fasting, the animals were administered with OG253 capsules three times per day (TID) at intervals of 8 h *via* oral gavage. All animals were observed for clinical signs including mortality and morbidity. Animals were observed at 1, 2, 4, 8, and 12 h post dosage and then twice daily for a total of 5 consecutive days. Food consumption and body weight (BW) were measured daily. Each cage contained a consecutive average of pre-weighed food daily and topped off to 30 g each day. Prior to weighing food, the cage was checked for any feed pellets that may have fallen from the wire rack but not eaten, prior to assessing the weight of remaining food. Animals were sacrificed on day 5.

2.4. Seven-days tolerability and toxicokinetics

Eight to ten weeks old Wistar Han rats (20 male and 20 female rats) were randomly assigned to 6 study groups as outlined in Table 2. Body weights of male and female rats varied widely. The mode of administration, animal welfare parameters, body weight measurements, food consumption measurements, cage side observations, and clinical observations were identical as in the one-day study presented above. Animals were orally gavaged TID (8 h apart) daily for 7 consecutive days with either placebo capsules of OG253 capsules. There were 6 animals in group T1-T4 ($N = 6$, 3 each from both sexes) and 8 animals ($N = 8$, 4 each from both sexes) in group TK-1-TK2 (Table 2). Animals in the groups were orally gavaged with either placebo capsules or OG253 capsules as described in Table 2.

Whole blood was collected in tubes containing K_2EDTA by retro-orbital sinus puncture prior to dosing (day-0) and on day-1 and day-7 from animals in the TK-1 and TK-2 (cohorts of 2 animals/sex; 2 cohorts bled three times to equal six time points: 1, 2, 4, 12, 20, and 24 h). Plasma was separated, stored and shipped frozen for bioanalytical testing. At termination on day-8, animals were euthanized by isoflurane inhalation and necropsied. Blood was collected and used for clinical chemistry (300 µL of serum), CBC with differential count (300 µL of whole blood in K_2EDTA-treated tubes) and coagulation (300 µL of citrate-treated plasma) assays. 500 µL of urine was collected prior to termination.

Hematology, coagulation, clinical chemistry, and urinalysis evaluations were conducted at Charles River RADS BioAssay Sevices, Willmington, MA, USA. Hematological parameters were assessed using Advia 120 Hematology System, Siemens Healthineers, Hoffman Estates, IL, USA. Clinical chemistry parameters assays were evaluated using Olympus AU400 Chemistry Analyzer, Bellport, NY, USA. Coagulation parameters were assesses using a fully automatic coagulation analyzer (STAGO, France). Urinalysis was evaluated using Siemens Clinitek 500, Siemens Healthineers, Malvern, PA, USA.

At study termination, a complete necropsy was performed on animals in the 7-days tolerability study. Microscopic examination of the cecum, colon, rectum, duodenum, ileum, and jejunum was conducted for all animals in the group T1-T4. Briefly, tissues were fixed in 10% neutral buffered formalin. The tissues were paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E) at Charles River Laboratories (Fredrick, MD, USA). Histopathology evaluation of the H&E stained slides was conducted at Charles River Laboratories (Durham, NC, USA). The tissue sections were evaluated using light microscopy by a board-certified veterinary pathologist.

2.5. Quantitation of OG253 in plasma

Quantitation of OG253 in rat plasma was performed at AIT Biosciences (Indianapolis, IN, USA) by LC-MS/MS as previously reported (Pulse et al., 2019). Briefly, a 50 µL sample is aliquoted into a pre-conditioned 96-well solid phase extraction (SPE) plate and mixed with SM270 internal standard (a closely related lanthipeptide). The samples were washed, eluted and evaporated to dryness before being reconstituted for analysis by LC-MS/MS. Results were quantified by extrapolation from an OG253 calibration curve prepared in a sample matrix. The research method has been qualified with a linear range of 10–10,000 ng/mL, with a lower limit of quantification of 10 ng/mL.

2.6. Data analysis

Data were analyzed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA) and presented as Mean ± SD. Statistical significance was determined by unpaired *t*-test (two-tailed). Differences and associations were considered statistically significant where $P < .05$.

Table 2
7-day tolerability and toxicokinetics study design of rats orally gavaged for 7 consecutive days (TID, 8 h apart) with placebo or OG253 capsules.

Group	N and sex	Treatment	Number of capsules/dose	Dosing interval	Daily dose (mg/kg)
T-1	6 (3 M and 3 F)	Placebo	1	8 hourly (TID)	0
T-2	6 (3 M and 3 F)	OG253 low dose	1	8 hourly (TID)	26.6
T-3	6 (3 M and 3 F)	OG253 mid dose	1	8 hourly (TID)	106
T-4	6 (3 M and 3 F)	OG253 high dose	2	8 hourly (TID)	425.7
TK-1	8 (4 M and 4 F)	OG253 mid dose	1	8 hourly (TID)	106
TK-2	8 (4 M and 4 F)	OG253 high dose	2	8 hourly (TID)	425.7

After overnight fasting, rats were orally gavaged with either placebo or OG253 capsules three times per day (TID, at intervals of 8 h) for 7-consecutive days. Body weights and food consumption of rats were measured daily.
Rats were euthanized on day 8. M denotes male rats. F denotes female rats.

3. Results

3.1. Characterization of test compounds

Lyophilized OG253 was produced as a trifluoroacetate salt, as previously described (Kers et al., 2019). The purity and identity of OG253 were characterized and confirmed using several analytical methods including HPLC and LC/MS. The purity of OG253 was determined at > 90% by HPLC and the identity of the product peak was confirmed by LC/MS (data not shown). Elemental analysis was used to calculate net peptide contents from lyophilized peptide powder. OG253 contained 72% of net peptide content by mass following a modified method of Vemuri (Vemuri, 2005) (data not shown). Fluorine (^{19}F) NMR was used to calculate the amount of trifluoroacetate counter-ion and it was found that OG253 had 6.3 M equivalent of trifluoroacetate counter ion (data not shown). The primary sequence was confirmed by extended NMR study (data not shown).

3.2. One-day acute tolerability of OG253 capsules

The experimental scheme of one-day tolerability study is shown in Table 1. During the clinical observation period, rats showed normal behavior without any morbidity or mortality. There were no statistical differences in body weight and food consumption of rats administered with three different doses of OG253 capsules (Fig. S1).

3.3. Seven-days tolerability and toxicokinetics of OG253 capsules

3.3.1. Clinical observations

All three doses of OG253 capsules were generally well tolerated with no mortality and morbidity. There was no significant change in the body weight and food consumption with seven-days of OG253 capsule administration compared to placebo administered rats (Fig. 3). OG253 capsule administration did not alter organ weights compared to placebo-administered rats (Table S1). Body weights of male rats were significantly higher than that of female rats when measured prior to the dosing of Placebo/OG253 capsules (Fig. S2). Male rats weighted on average 293.6 ± 1.579 g and female rats weighed an average of and 216.0 ± 1.447 g ($P < .0001$).

3.3.2. Organ weights

There was no significant change in the weight of organs, glands and reproductive organs of animals administered with OG253 capsules compared to placebo capsule administered animals (Table S1). The weight of liver, brain, heart, kidneys, spleen, duodenum, cecum, ileum, jejunum, rectum, adrenal glands, thymus, thyroid, testes, epididymis, prostate, ovaries and uterus are shown in Table S1.

3.3.3. Hematology, coagulation and clinical chemistry

OG253 capsule administration did not change red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT) and platelet volume (MPV) as compared to placebo administered rats (Fig. S3). However, there was a statistically significant ($P = .0351$) increase in the white blood cell (WBC) count of female rats in the OG253 low dose treated group compared to placebo capsule treated rats (Fig. S3). OG253 treatment did not modulate prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen (Fib) levels as compared to placebo administered rats (Fig. S4).

OG253 capsule administration did not alter alanine aminotransaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALK), glucose (GLU), phosphate (PHOS), calcium (CA), total bilirubin (TBIL), total protein (TP) and albumin (ALB), globulin (GLOB), albumin/globulin Ratio (A/G ratio), blood urea nitrogen (BUN), creatinine (CREAT), sodium (Na), potassium (K), chlorine (Cl) and sodium potassium ratio (Na/K ratio) as compared to placebo-treated rats (Fig. S5).

OG253 capsule treatment showed a dose-response effect in reducing serum triglycerides and cholesterol (Fig. 4A and B) as compared to placebo capsule administered animals. A statistically significant decrease in the serum triglyceride level was observed in the animals administered with OG253 mid and high doses ($P = .0413$ and 0.0002, respectively), as compared to placebo administered rats. Overall, there was a 61.38% (55.62 and 67.56% in male and female rats, respectively) reduction in the serum triglyceride of rats administered with OG253 high dose as compared to placebo-administered rats (Fig. 4A). Overall, there was a 29.89% (31.97 and 28.00% in male and female rats, respectively) reduction in the serum cholesterol of animals administered with OG253 high dose as compared to placebo administered rats ($P = .0002$, Fig. 4B). Statistically significant reduction in both serum triglyceride and cholesterol was observed in animals administered with OG253 high dose compared to OG253 low dose ($P = .0031$ and 0.0077, respectively, Fig. 4A and B).

3.3.4. Urinalysis

There was no change in the urine pH of OG253 administered animals compared to placebo-administered rats (Fig. S6).

3.3.5. Histopathology

There were no histopathological evidence of OG253 treatment-related effect on rat cecum, colon, ilium, and duodenum (Table S2). OG253 treatment-related findings were confined to the rectum and consisted of glandular atrophy in two male rats dosed at OG253 high dose. The change was characterized by a multifocal absence of glands within the rectal mucosa, a reduction in the overall height of the mucosa, by a loss of glandular epithelium and mucous goblet cells within the rectal mucosa and by a reduction in the size and number of mucous goblet cells within the epithelium. The intervening stroma of the lamina propria appeared increased due to condensation from loss of mucosal glands. Severity was assigned based on the total area affected. The change was more widely disseminated in one male rat at OG253 high dose, affecting multiple areas throughout the section and was considered as mild glandular atrophy. In another male rat, the finding was apparent multifocal, but did not affect the total area and was therefore considered as minimal glandular atrophy (Table S2). The toxicologic significance of the glandular atrophy is questionable due to their morphologic similarity to tissue handling artifacts noted in other sections as well as no evidence of glandular atrophy in other regions of the intestinal tract.

3.3.6. Bioavailability of OG253 in plasma

OG253 was not detected in the plasma samples collected at various time points (0, 1, 2, 4, 12, 20 and 24 h) after the administration of mid and high doses of OG253 (Data not shown). The results indicate that OG253 was not readily absorbed into the bloodstream but rather remains localized in the gastrointestinal tract.

4. Discussion

CDAD causes significant morbidity and mortality (Dieterle et al., 2019). With an incidence reaching nearly 500,000 cases annually in the United States, there is a substantial need for the development of therapies against CDAD. Previous studies unveiled specific amino acid substitutions of MU1140 that improved the physicochemical and/or pharmacological properties of the molecule for the treatment of CDAD. The engineering and triage-testing of over 400 single amino acid substitutions identified a small subset of variants with improved potency, adequate stability and solubility in bio-relevant fluids, low levels of overall toxicity *in vitro* and appropriate levels of efficacy *in vivo*, as evidenced by a cannulated model of the Syrian Hamster CDAD model (Kers et al., 2018b; Kers et al., 2019). Interestingly, the lantibiotic

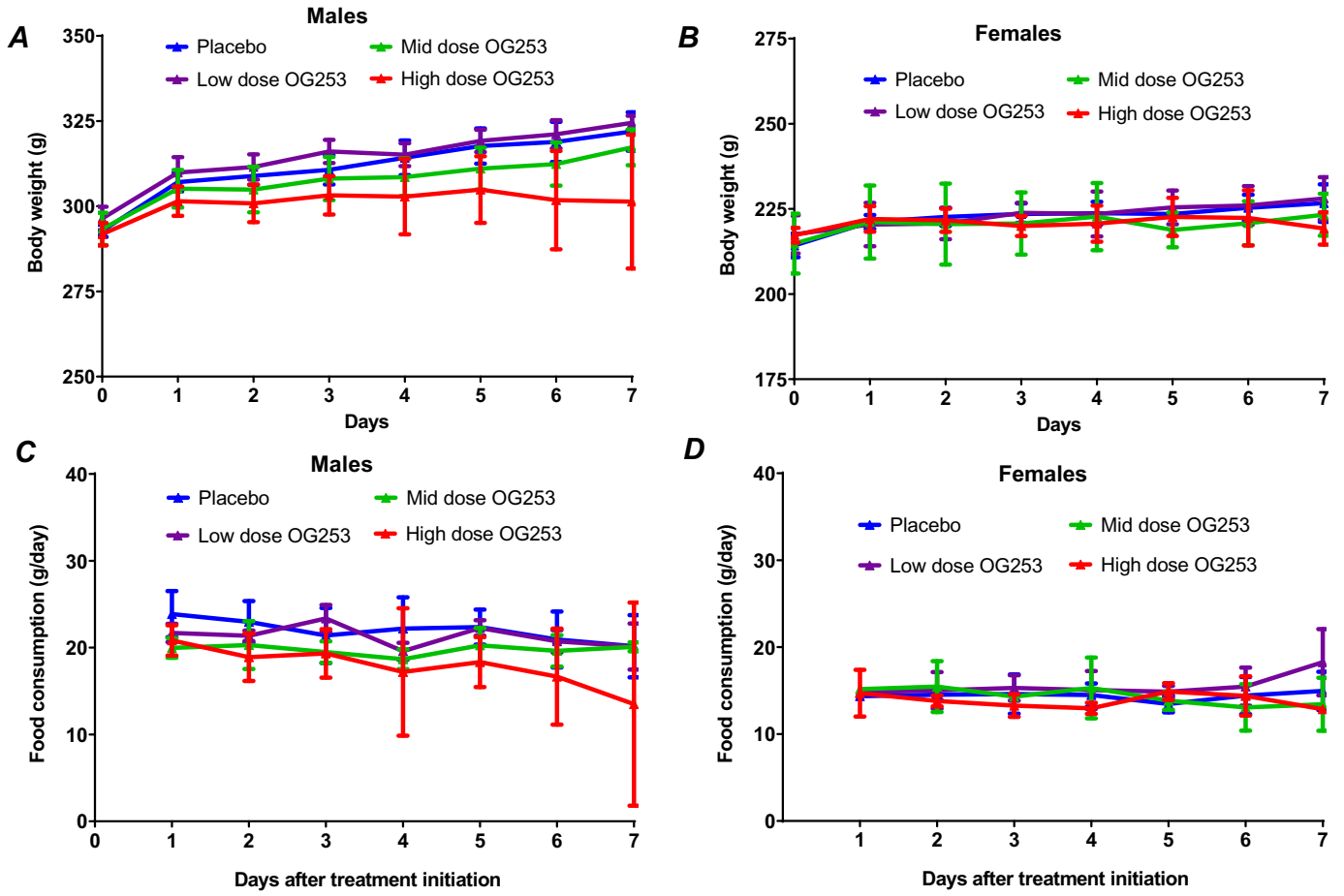

Fig. 3. Effect of Placebo or OG253 capsule administration on the body weight and food consumption of rats in the 7-days study. Rats were administered with either placebo or OG253 capsules as described in the Materials and Methods. (A) Body weight curves of male rats, (B) body weight curves of female animals, (C) food consumption of male rats and (D) food consumption of female rats. The administration of OG253 capsules did not significantly modulate body weight or food consumption of rats compared to placebo-capsule administrated rats. Data represent Mean ± SD. $N = 3$ animals/sex/group.

OG253 (MU1140 variant Phe1Ile) performed best in preliminary efficacy assessment testing, despite the presence of a trypsin/chymotrypsin digestion site on residue Arg13 (Kers et al., 2018b). While the stability of this lantibiotic in a fasted state simulated gastric fluid (FaSSGF) was high under the conditions tested, its half-life in s fasted state simulated intestinal fluid (FaSSIF) demonstrated sensitivity to proteolytic degradation under conditions tested. Other variants (*e.g.* OG191, MU1140-Arg13Asn) with substitutions at Arg13 that are less sensitive to trypsin/chymotrypsin digestion presented with a half-life > 240 min, while the half-life of OG253 in FaSSIF was only 95 min. Nevertheless, OG253 delivered to cannulated Syrian Hamsters with CDAD ultimately emerged as the lead compound of this study, based on its superior efficacy *in vivo* and the apparent lack of relapse over the 21 days of this animal study (Kers et al., 2018b). An enteric-coated capsule of OG253 was formulated in an attempt to specifically deliver this lantibiotic to the distal portion of the small intestine. Enteric-coated OG253 capsules were used to evaluate the toxicokinetics and to estimate the MTD following one-day and seven-day repeated daily oral dosage in Wistar Han rats.

OG253 high dose was selected based on the maximum capsule volume for oral gavage (size-9), the maximum API fill per capsule (18 mg), and the maximum number of capsules (2 capsules) that can be gavaged orally to each animal at each time point, following a three times a day (TID) dosing regimen. This resulted in a high dose of 108 mg/day (425.7 mg/kg/day), based on the average baseline body weight of 253.7 g per rats. The mid dose (27 mg/day or 106 mg/kg/day) was chosen as a four-fold decrease of the high dose, and the low dose (6.75 mg/day or 26.6 mg/kg/day) was chosen as a four-fold decrease from the mid dose. Clinical signs, body weights, and food consumption remained normal throughout the one-day regimen supported the design of the seven-day repeated dose protocol.

In the second phase of this study, the same overall study design of one-day acute tolerability study was used except that the study duration was seven-days and the study included cage-side monitoring, detailed clinical observations, clinical pathology, functional observations, complete necropsy, urinalysis, hematology, clinical chemistry, coagulation, and standard histopathological panel focused on the GI tract for evidence of local toxicities. In addition, toxicokinetics (TK) arm was included in the study groups to specifically assess the levels of potential absorption of OG253 through the vasculature. Minimal toxicity was expected in this protocol, considering the structure and chemical nature of lanthipeptide, the oral route of administration of OG253 and previous reports from *in vitro* cytotoxicity and off-target profiling (Kers et al., 2018b). Consistent with these data, there were no significant differences in the body weight, food consumption and weights of organs of animals administered with OG253 capsules as compared to placebo-treated animals. OG253 treatment did not affect any of the hematological and clinical chemistry parameters. Histopathological findings associated with the administration of OG253 were confined to the rectum, resulting in glandular atrophy in two out of six male rats dosed at high dose (Table S2).

OG253 was undetectable in the rat plasma samples collected at various time points (0, 1, 2, 4, 12, 20 and 24 h) after the administration of mid and high doses of OG253. The samples were tested using a

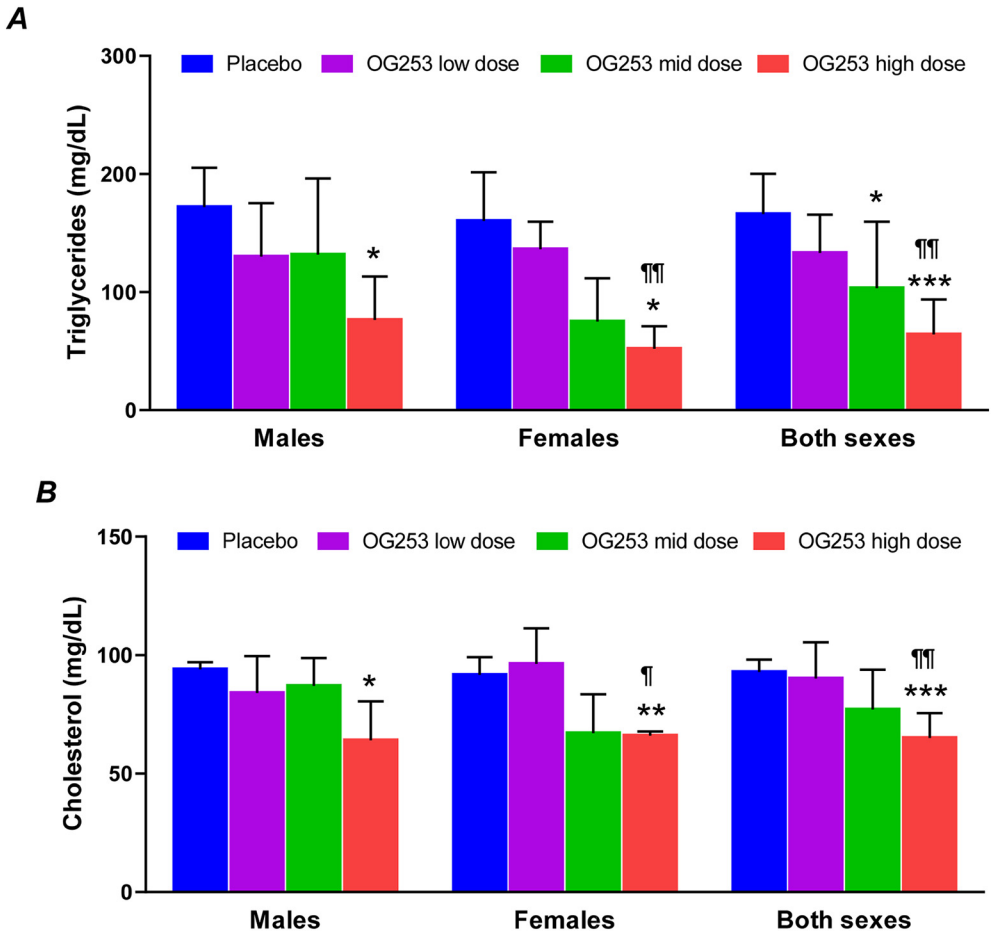

Fig. 4. Effect of OG253 capsule administration on the serum triglyceride and cholesterol levels of rats. Rats were administered with either placebo or OG253 capsules as described in the Materials and Methods. (A) Both the mid and high dose of OG253 treatment resulted in significantly decreased triglyceride levels compared to placebo treated animals (*P = .0413 and ***P = .0002). Significantly decreased triglyceride level was observed in the high dose OG253 treated animals compared to low dose OG253 (¶¶P = .0031). In both sexes together, there was a 61.38% decrease in triglycerides at the OG253 high dose compared to placebo. High dose of OG253 treated animals resulted in 55.62% and 67.56% reduction of triglycerides in the male and female rats, respectively (*P = .0288 and *P = .0144, respectively). Significantly decreased triglyceride level was observed in the high dose OG253 treated female rats compared to low dose OG253 treated female rats (¶¶P = .0083). (B) High dose OG253 treatment resulted in significantly reduced serum cholesterol levels (***P = .0002) compared to placebo-treated animals. In both sexes together, there was an overall 29.98% decrease in the cholesterol of animals administered with high dose OG253 compared to placebo-treated rats. Significantly decreased cholesterol level was observed in the high dose OG253 treated animals compared to low dose OG253 (¶¶P = .0077). High dose OG253 treatment resulted in 31.91% and 28.00% reduction of cholesterol in male and female rats, respectively (*P = .0360 and **P = .0045, respectively). Significantly decreased cholesterol level was observed in the high dose OG253 treated female rats compared to low dose OG253 treated female rats (¶P = .0254). Data represent Mean ± SD. N = 6 (3 animals/sex/group).

method sensitive to a LLOQ of 20 ng/mL, indicating that OG253 was not absorbed into the bloodstream. This finding was expected considering that the molecular weight of OG253 (~2.2 kDa) far exceeds the maximum molecular weight cutoff (~500 Da) of compounds that are typically transported across the GI barrier (Veber et al., 2002). Further study is required to determine if *C. difficile* infection (often characterized by "leaky guts") affect the bioavailability of OG253.

Our finding of a dose-dependent effect of OG253 in reducing serum triglyceride and cholesterol was unexpected (Fig. 4). High dose OG253 (425.7 mg/kg/day) treatment resulted in a statistically significant reduction of both serum triglyceride and cholesterol in both sexes of rats (Fig. 4A and B). A direct effect of antibiotics on cholesterol and triglycerides has previously been reported (Steiner et al., 1961; Jenkins et al., 2005), and it appears that the amplitude of the effect observed herein is consistent to the dose of the drug. A recent study reported that amoxicillin antibiotic treatment (20.82 mg/day for six consecutive days) significantly reduced both serum triglyceride and cholesterol levels in healthy neonatal pigs compared to saline-treated neonatal pigs (Wan et al., 2019). Decreases in the levels of serum cholesterol was initially fortuitously discovered upon treatment with neomycin at doses between 0.5 and 2 g/day (or ~33 mg/kg/day, assuming an average weight of an adult human is 60 kg), and observed a decrease up to 25% in the levels of serum cholesterol (Steiner et al., 1961). Other antibiotics were also found to exert a similar effect, albeit of lesser amplitude, and included kanamycin, streptomycin, bacitracin, aureomycin, achromycin, but not chloromycetin, mycostatin or penicillin (Steiner et al., 1961). More contemporary reports have noticed the same effect with other antimicrobials used at therapeutic doses, including metronidazole and ciprofloxacin, at therapeutic doses of ~17 mg/kg/day (Jenkins et al., 2005). In the latter example, a 10-days metronidazole treatment led to a 14% decrease in serum cholesterol. Interestingly, the authors also correlated this decrease with an increase in bifidobacteria (P = .029). Gut microbiota has been known to modulate bile acid (BA) pool size and composition. BA biotransformation is involved in the regulation of dietary lipid absorption and can act as signaling molecules, modulating cholesterol and triglyceride metabolism (Houten et al., 2006; Ridlon et al., 2006). Therefore, any perturbation of the flora may result in decreased dietary lipid absorption and modulate cholesterol and triglyceride metabolism and could serve as a basis to explain the decrease in cholesterol and triglycerides observed in the current study.

Another possibility is that a large lipophilic region of the lantibiotic may have been binding to the lipophilic regions in cholesterol, in a process similar to chelation, which may have resulted in retaining fats in the GI tract (Genuis et al., 2013). There is a striking structural similarity between the pyrophosphate moiety of Lipid II and several intermediates in the isoprenoid biosynthesis pathway (Chugunov et al., 2013). Isoprenoids constitute one of the most diverse classes of natural products. As a compound class, they are essential to basic metabolic processes including cell-wall biosynthesis, post-translational protein modifications, and signaling. Not surprisingly, the isoprenoid pathway is an essential metabolic pathway present in eukaryotes, archaea and some bacteria (Buhaescu and Izzedine, 2007). The pathway produces two five-carbon building blocks called isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are used to make isoprenoids, a diverse class of over 35,000 biomolecules such as cholesterol, vitamin K, coenzyme Q10, and all steroid hormones (Holstein and Hohl, 2004; Heuston et al., 2012). The mevalonate pathway is best

known as the target of statins, a class of cholesterol-lowering drugs (Goldstein and Brown, 1990). Since OG253 is not absorbed systemically, its effect on cholesterol and triglycerides would presumably be confined to the intestinal compartment by a direct antimicrobial effect. It remains unclear whether or not the observed lowering effect of cholesterol and triglycerides is a transient effect that can be reversed upon cessation, as previously observed with different classes of antibiotics (Steiner et al., 1961). Additional experiments are underway to characterize this observation and substantiate the mechanism(s) involved.

In conclusion, enteric-coated OG253 has a favorable safety profile in rats. All three doses of OG253 capsules, up to the 108 mg/day (425.7 mg/kg/day), were generally well tolerated with no mortality and morbidity. There were no OG253-related effects on body weight and food consumption. The treatment did not change hematological and coagulation parameters or urine pH, compared to placebo administered rats. Other than a significant reduction in serum cholesterol and triglyceride levels, clinical chemistry parameters were not relatively influenced by OG253. The absence of OG253 in the plasma indicate that OG253 is not absorbed into the blood from the rat gastrointestinal tract. Overall, our results suggest that OG253 is safe and may serve as a lead compound for further preclinical studies.

Conflict of interest

NVR, JAK, and SM were Intrexon employees during the data collection and initial data analysis. JAK is a stockholder of Intrexon. AWD was an Oragenics stockholder and employee during data collection and initial data analysis. JHP and MH are employees and stockholders of Oragenics during data collection, analysis, and writing of the manuscript.

Acknowledgments

The authors acknowledge Eryl Sharp, Sheela Muley, Melissa Mayo, Jeffrey Colbeck, Albert G. Fosmoe, II, Bruce Tilley, Gabriela Philips, Mathoor Sivaram, Joel Ngoje, Fernando Anasco, Zaxton Lamon, Emily Richeson and Vicky DaSilva for their contribution to the to the production, purification and testing/characterization of Mutacin 1140 variants. MH is grateful to Charles River Labs (Nataliya Sadekova, Michelle King, Kevin Norton, and Andreanne Morency), AIT Biosciences (Brad King), Emerson Resources (Nicolas Kirkland, Rob Tuohy and Aaron Barkley) and Synergy Partners (Shelley Ching and Jim MacDonald) and Jin Xu (UMass, Lowell) for their help in the design and execution of several of the animal studies, characterization and analytical assays reported in this work. The authors are grateful to David Blum and Ron Garisson (Bioexpression and Fermentation Facility at the University of Georgia) for performing fermentation work, and Carl Lawton (University of Massachusetts, Lowell) and Kevin Millsap for critical input on fermentation optimization. JHP is grateful to Ion Ghiviriga,

NMR facility, University of Florida and Patricia Y. Coxon, Peptide International for extended NMR study and elemental analysis, respectively. The authors are grateful to Tahnia Martin and Rahim Johnson for their help in animal experiments. MH and JHP are grateful to Alan Joslyn for the critical review of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.taap.2019.04.019.

References

Boakes, S., Weiss, W.J., Vinson, M., Wadman, S., Dawson, M.J., 2016. Antibacterial activity of the novel semisynthetic lantibiotic NVB333 in vitro and in experimental infection models. J. Antibiot. (Tokyo) 69, 850–857.

Buhaescu, I., Izzedine, H., 2007. Mevalonate pathway: a review of clinical and therapeutical implications. Clin. Biochem. 40, 575–584.

Chatterjee, C., Paul, M., Xie, L., van der Donk, W.A., 2005. Biosynthesis and mode of action of lantibiotics. Chem. Rev. 105, 633–684.

Chen, S., Wilson-Stanford, S., Cromwell, W., Hillman, J.D., Guerrero, A., Allen, C.A., Sorg, J.A., Smith, L., 2013. Site-directed mutations in the lanthipeptide mutacin 1140. Appl. Environ. Microbiol. 79, 4015–4023.

Chugunov, A., Pyrkova, D., Nolde, D., Polyansky, A., Pentkovsky, V., Efremov, R., 2013. Lipid-II forms potential "landing terrain" for lantibiotics in simulated bacterial membrane. Sci. Rep. 3, 1678.

Di Bella, S., Ascenzi, P., Siarakas, S., Petrosillo, N., di Masi, A., 2016. *Clostridium difficile* toxins A and B: insights into pathogenic properties and extraintestinal effects. Toxins (Basel) 8, 134.

Dieterle, M.G., Rao, K., Young, V.B., 2019. Novel therapies and preventative strategies for primary and recurrent *Clostridium difficile* infections. Ann. N. Y. Acad. Sci. 1435, 110–138.

Gauvin, D.V., Dalton, J.A., Harter, M.L., Holdsworth, D., May, J., Tapp, R., Zimmermann, Z.J., Kilgus, Q., Baird, T.J., 2019. Relative equivalence of CNS safety (FOB) assessment outcomes in male and female Wistar-Han and Sprague-Dawley rats. J. Pharmacol. Toxicol. Methods 95, 2–11.

Genuis, S.J., Sears, M.E., Schwalfenberg, G., Hope, J., Bernhoft, R., 2013. Clinical detoxification: elimination of persistent toxicants from the human body. Sci. World J. 2013, 238347.

Ghobrial, O.G., Derendorf, H., Hillman, J.D., 2009. Pharmacodynamic activity of the lantibiotic MU1140. Int. J. Antimicrob. Agents 33, 70–74.

Ghobrial, O., Derendorf, H., Hillman, J.D., 2010. Pharmacokinetic and pharmacodynamic evaluation of the lantibiotic MU1140. J. Pharm. Sci. 99, 2521–2528.

Goldstein, J.L., Brown, M.S., 1990. Regulation of the mevalonate pathway. Nature 343, 425–430.

Grasemann, H., Stehling, F., Brunar, H., Widmann, R., Laliberte, T.W., Molina, L., Doring, G., Ratjen, F., 2007. Inhalation of Moli1901 in patients with cystic fibrosis. Chest 131, 1461–1466.

Heuston, S., Begley, M., Gahan, C.G., Hill, C., 2012. Isoprenoid biosynthesis in bacterial pathogens. Microbiology 158, 1389–1401.

Hillman, J.D., Johnson, K.P., Yaphe, B.I., 1984. Isolation of a *Streptococcus mutans* strain producing a novel bacteriocin. Infect. Immun. 44, 141–144.

Hillman, J.D., Novak, J., Sagura, E., Gutierrez, J.A., Brooks, T.A., Crowley, P.J., Hess, M., Azizi, A., Leung, K., Cvitkovitch, D., Bleiweis, A.S., 1998. Genetic and biochemical analysis of mutacin 1140, a lantibiotic from *Streptococcus mutans*. Infect. Immun. 66, 2743–2749.

Holstein, S.A., Hohl, R.J., 2004. Isoprenoids: remarkable diversity of form and function. Lipids 39, 293–309.

Houten, S.M., Watanabe, M., Auwerx, J., 2006. Endocrine functions of bile acids. EMBO J. 25, 1419–1425.

Jenkins, D.J., Kendall, C.W., Hamidi, M., Vidgen, E., Faulkner, D., Parker, T., Irani, N., Wolever, T.M., Fong, I., Kopplin, P., Connelly, P.W., Onderdonk, A., Rao, A.V., 2005. Effect of antibiotics as cholesterol-lowering agents. Metabolism 54, 103–112.

Kers, J.A., DeFusco, A.W., Park, J.H., Xu, J., Pulse, M.E., Weiss, W.J., Handfield, M., 2018a. OG716: designing a fit-for-purpose lantibiotic for the treatment of *Clostridium difficile* infections. PLoS One 13, e0197467.

Kers, J.A., Sharp, R.E., Defusco, A.W., Park, J.H., Xu, J., Pulse, M.E., Weiss, W.J., Handfield, M., 2018b. Mutacin 1140 lantibiotic variants are efficacious against *Clostridium difficile* infection. Front. Microbiol. 9, 415.

Kers, J.A., Sharp, R.E., Muley, S., Mayo, M., Colbeck, J., Zhu, Y., DeFusco, A.W., Park, J.H., Handfield, M., 2019. Blueprints for the rational design of therapeutic mutacin 1140 variants. Chem. Biol. Drug Des. 92, 1940–1953.

Knerr, P.J., van der Donk, W.A., 2012. Discovery, biosynthesis, and engineering of lantipeptides. Annu. Rev. Biochem. 81, 479–505.

Leffler, D.A., Lamont, J.T., 2015. *Clostridium difficile* infection. N. Engl. J. Med. 372, 1539–1548.

Lessa, F.C., Mu, Y., Bamberg, W.M., Beldavs, Z.G., Dumyati, G.K., Dunn, J.R., Farley, M.M., Holzbauer, S.M., Meek, J.I., Phipps, E.C., Wilson, L.E., Winston, L.G., Cohen, J.A., Limbago, B.M., Fridkin, S.K., Gerding, D.N., McDonald, L.C., 2015. Burden of *Clostridium difficile* infection in the United States. N. Engl. J. Med. 372, 825–834.

Magill, S.S., Edwards, J.R., Bamberg, W., Beldavs, Z.G., Dumyati, G., Kainer, M.A., Lynfield, R., Maloney, M., McAllister-Hollod, L., Nadle, J., Ray, S.M., Thompson, D.L., Wilson, L.E., Fridkin, S.K., Emerging Infections Program Healthcare-Associated, I, Antimicrobial Use Prevalence Survey, T, 2014. Multistate point-prevalence survey of health care-associated infections. N. Engl. J. Med. 370, 1198–1208.

Martin, J.S., Monaghan, T.M., Wilcox, M.H., 2016. *Clostridium difficile* infection: epidemiology, diagnosis and understanding transmission. Nat. Rev. Gastroenterol. Hepatol. 13, 206–216.

Ofosu, A., 2016. *Clostridium difficile* infection: a review of current and emerging therapies. Ann. Gastroenterol. 29, 147–154.

Ongey, E.L., Yassi, H., Pflugmacher, S., Neubauer, P., 2017. Pharmacological and pharmacokinetic properties of lanthipeptides undergoing clinical studies. Biotechnol. Lett. 39, 473–482.

Pharmacopeia, US, 2012. Disintegration. http://www.drugfuture.com/Pharmacopoeia/usp35/PDF/0293-0295%20[701]%20DISINTEGRATION.pdf.

Pharmacopeia., US, 2011. Uniformity of Dosage Units. http://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q0304_stage_6_monograph_25_feb_2011.pdf (Stage 6 Harmonization).

Pharmacopeia., US, 2012. Weight Variation of Dietary Supplements. https://www.usp.org/sites/default/files/usp/document/products-services/products/2015-dsc-vol-1-table-of-contents-ref-standard-index.pdf (Accessed 25 September 2017).

Pulse, M.E., Weiss, W.J., Kers, J.A., DeFusco, A.W., Park, J.H., Handfield, M., 2019. Pharmacological, toxicological and dose-range assessment of OG716, a novel lantibiotic for the treatment of *Clostridium difficile* associated infection (CDI). Antimicrob. Agents Chemother. 63 (pii: e01904-18).
Ridlon, J.M., Kang, D.J., Hylemon, P.B., 2006. Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. 47, 241–259.
Ross, A.C., Vederas, J.C., 2011. Fundamental functionality: recent developments in understanding the structure-activity relationships of lantibiotic peptides. J. Antibiot. (Tokyo) 64, 27–34.
Sahl, H.G., Jack, R.W., Bierbaum, G., 1995. Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. Eur. J. Biochem. 230, 827–853.
Sandiford, S.K., 2015. Perspectives on lantibiotic discovery - where have we failed and what improvements are required? Expert Opin. Drug Discovery 10, 315–320.
Smith, L., Hasper, H., Breukink, E., Novak, J., Cerkasov, J., Hillman, J.D., Wilson-Stanford, S., Orugunty, R.S., 2008. Elucidation of the antimicrobial mechanism of mutacin 1140. Biochemistry 47, 3308–3314.
Steiner, A., Howard, E., Akgun, S., 1961. Effect of antibiotics on the serum cholesterol concentration of patients with astherosclerosis. Circulation 24, 729–735.
Theuretzbacher, U., Gottwalt, S., Beyer, P., Butler, M., Czaplewski, L., Lienhardt, C., Moja, L., Paul, M., Paulin, S., Rex, J.H., Silver, L.L., Spigelman, M., Thwaites, G.E., Paccaud, J.P., Harbarth, S., 2019. Analysis of the clinical antibacterial and antituberculosis pipeline. Lancet Infect. Dis. 19, e40–e50.
Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 45, 2615–2623.
Vemuri, S., 2005. Comparison of assays for determination of peptide content for lyophilized thymalfasin. J. Pept. Res. 65, 433–439.
Wan, J.J., Lin, C.H., Ren, E.D., Su, Y., Zhu, W.Y., 2019. Effects of early intervention with maternal fecal bacteria and antibiotics on liver metabolome and transcription in neonatal pigs. Front. Physiol. 10, 171.